SCIENTIFIC VALIDATION OF ANTI-DIABETIC, ANTI-DYSLIPIDEMIC AND ANTI-OXIDANT ACTIVITIES OF SIDDHA HERBAL FORMULATION *"ELATHY URUNDAI"* IN IN-VIVO AND IN-VITRO MODELS

The dissertation Submitted by

Dr. B. KARVANTHAN

Reg.No. 321412107

Under the Guidance of

Dr. V. VELPANDIAN, M.D(S), Ph.D.,

Dissertation submitted to

THE TAMILNADU DR. MGR MEDICAL UNIVERSITY

CHENNAI-600032

In partial fulfilment of the requirements

For the award of the degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH-II-GUNAPADAM

POST GRADUATE DEPARTMENT OF GUNAPADAM

GOVERNMENT SIDDHA MEDICAL COLLEGE

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Scientific Validation of Anti- Diabetic, Anti-Dyslipidemic and Anti-Oxidant Activities of Siddha Herbal Formulation *"ELATHY URUNDAI"*** is a bonafide and genuine research work carried out by me under the guidance of **Dr.V.Velpandian, M.D(S), Ph.D.,** Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date: **Signature of the Candidate**

Place: Chennai **Dr.B.Karvanthan**

GOVT. SIDDHA MEDICAL COLLEGE, ARUMBAKKAM, CHENNAI-106

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled "**Scientific Validation of Anti- Diabetic, Anti-Dyslipidemic and Anti-Oxidant Activities of Siddha Herbal Formulation "Elathy Urundai"** is submitted to the Tamilnadu Dr.M.G.R.Medical University in partial fulfillment of the requirements for the award of degree of M.D (Siddha) is the bonafide and genuine research work done by **Dr.B.Karvanthan** Under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

Date: **Signature of the Guide**

Place: Chennai

GOVT. SIDDHA MEDICAL COLLEGE, ARUMBAKKAM, CHENNAI-106

ENDORSEMENT BY THE HOD AND PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **"Scientific Validation of Anti- Diabetic, Anti-Dyslipidemic and Anti-Oxidant Activities of Siddha Herbal Formulation "Elathy Urundai"** is a bonafide work carried out by **Dr.B.Karvanthan** under the guidance of **Dr.V.Velpandian, M.D(S), Ph.D.,** Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Chennai - 106.

Signature of the HOD **Signature of the Principal**

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ABBREVIATION

AGE	**Advanced Glycation End Product**
ALT	**Alanine Aminotransferase**
AST	**Aspartate Aminotransferase**
ATP	**Adenosine Triphosphate**
BUN	**Blood Urea Nitrogen**
CPCSEA	**Committee for the Purpose of Control and Supervision of Experiments on Animals.**
DNA	**Deoxyribonucleic Acid**
DTNB	**Dithionitro Bis – Benzoid acid**
ESR	**Erythrocyte Sedimentation Rate**
EU	***Elathy Urundai***
FTIR	**Fourier transform infra red spectrometer**
GDM	**Gestational Diabetes mellitus**
GLC	**Gas Liquid Chromatography**
GOT	**Glutamic Oxaloacetate Transaminase**
GPT	**Glutamate Pyruvate Transaminase**
GPx	**Glutathione Peroxidase**
GSH	**Glutathione**
Hb	**Haemoglobin**
HbA_1C	**Glycosylated Haemoglobin**
HDL	**High Density Lipoprotein**

HPTLC	**High Performance Thin Layer Chromatography**
IAEC	**Institutional Animal Ethical Committee**
IDDM	**Insulin Dependent Diabetes mellitus**
ICP	**Inductively Coupled argon Plasma**
ICPOES	**Inductively Coupled Plasma Optic Emission Spectrometry**
IU	**International Unit**
LDL	**Low Density Lipoprotein**
LMCV	**Lymphocytic Choriomeningitis Virus**
NIDDM	**Non Insulin Dependent Diabetes Mellitus**
NPH	**Neutral Protamine Hagedorn**
OECD	**Organization for Economic Co-operation and Development**
OES	**Plasma Emission Spectroscopy**
PCV	**Packed Cell Volume**
RBC	**Red Blood Corpuscles**
RNA	**Ribonucleic Acid**
SEM	**Scanning Electron Microscope**
STZ	**Streptozotocin**
TBA	**Thiobarbituric Acid**
TBARS	**Thiobarbituric Acid Reactive Substances**
TGL	**Triglyceride**
TLC	**Thin Layer Chromatography**
VLDL	**Very Low Density Lipoprotein**
WBC	**White Blood Corpuscles**

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INTRODUCTION

1. INTRODUCTION

Diabetes mellitus is a major lifestyle disorder, which is a problem faced majorly by the world today. WHO defines Diabetes mellitus as a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. It is characterized by the symptoms such as thirst, polyuria, blurring of vision and weight loss.

According to International Diabetes Federation (IDF) Report 2015,

- Worldwide - 450 million people have Diabetes and 318 million in pre-diabetic stage.
- India is in the second spot with 69.2 million people with Diabetes. India leads the word with largest number diabetics earning the name "Diabetes capital of the world"[1]

According to the latest reports from WHO, it is estimated that 415 million people suffered with Diabetes globally in 2015. In the year 2012 to 2015, an estimated 1.5 to 5.0 million deaths were caused by diabetes. WHO projects that diabetes will be the 7th leading cause of death in 2030 [2].

In 2014, India topped the world with the highest number of people with diabetes mellitus. It is predicted that by 2030, diabetes mellitus may affect up to 79.4 million individuals in India.[3]

The mortality rate was estimated 24 million persons with in India in 2000 and this number is predicted to rise to almost 70 million people by 2025[4].

Current treatment strategy for Diabetes mellitus includes conventional therapy with Insulin in case of Type-1 Diabetes and with wider combination of drugs in case of Type-2 Diabetes mellitus. Some of the commonly used drugs are Meglitinides, Sulfonylurea and Biguanides.

Meglitinides – a class of drug that act by promoting insulin secretion from pancreas. Among these Repaglinide(Prandin) and Nateglinide(Starlix) is particularly beneficial in lowering blood glucose after meals and does not tend to lower fasting

glucose levels to the same degree. The possible side effects of these drugs include sweating, confusion, tremors and coma.

Sulfonylurea drugs include glyburide (Diabeta), glipizide (Glucotrol) and glimepiride (Amaryl) – rapidly lower blood sugar, but can cause hypoglycemia. These drugs are to be avoided in patients with Sulfa allergy.

Metformin – has the ability to decrease glucose production by the liver. The possible side effects are loss of appetite and renal impairment [5].

In Siddha system Diabetes mellitus is referred as *Madhumegam*. Medical experts in Siddha medicine with exposure to modern science correlate *Madhumegam* to Diabetes mellitus. This is mainly because of the etymology that mellitus means sweetness which is *madhu*. The word '*Madhu*' means honey, the word 'Megam' in the Siddha system is releated to urinary disorder. Hence passing urine with sweetness is called 'Madhumegam'. The name Madhumegam is suitable for the symptoms of passing urine with sweetness. Extensive details of the urinary diseases which manifest as excessive urination or decreased urination is explained by Siddhar Therayar, one of the pioneers of the Siddha medical system, as Neerinai perukkal noi and Nerinai arukkal noi. Madhumegam is classified under "Neerinai perrukkal noi"

It is quoted as,

நீரிருவினைக் குணத்தை நீயறிவிரித்து சொல்வாம்

நீரினைப் பெருக்கலொன்று நீரினையருக்க லொன்று

நீரிழிவுடனே கொல்லும் நீர்க்கட்டு வினைகளென்று

நீணிலமுறைக் குமிந்த நீர்நிறைக் குணத்தைக் கேளாய்

\- தேரையர் மகாகரிசல்[6]

The sudden spurt in life style modification, ethical susceptibility and vast urbanization has made India the Diabetic capital of the World.

Siddhars have explained the 10 stages of Madhumegam called Avathai and also the symptoms in each stage by mentioning about medicines for Madhumegam , they have prescribed both herbal and mineral preparations.

The Siddha system of medicine has been in practice in our country since time unknown. The Siddha system of medicine is one of the ancient system of medicine. This system is having a specific character and having speciality that it not only cures the disease but also prevents the disease. This system of medicine is a complex of spirituality and healing technology that exploits herbal, metal, mineral substance, standing of course on tested theoretical foundations.

Siddhars were a disparate band of poet – philosophers with wide range of interests and capabilities. Their compositions are replete with philosophies of life which are unorthodox and radical in nature. Nevertheless, they are essentially men of medicine, and their philosophy is the philosophy of medicine. Their compositions comprised profound insights into securing the health of human body and mind as well. Their knowledge of the medical uses of a whole parts of herbs, metals and minerals is indeed so comprehensive and eclectic are their conceptions of medicine that they should not only make for a rewarding complement to all Synthetic drugs, but give a new perspective to our philosophy of healing.[7]

The Siddha system of medicine insists the use herbals first which is quoted as

"வேர் பாரு தழை பாரு மிஞ்சினக்கால்

மெல்ல மெல்ல பற்ப செந்தூரம் பாரே"

- கண்ணுசாமியம் பிள்ளை

This system has 64 different types of medications which includes internal as well as external medications. *Urundai* (pills) is one among the internal medicines. Siddha system offers cure for several diseases and also in the management of various incurable diseases. One such disease is Diabetes mellitus.

Elathy urundai is a poly herbal formulation comes under the classification of internal medicine is mentioned as a potent anti-diabetic drug in the Siddha classical literature ***Sarabendrar Vaidhiya Muraigal*** indicated for Diabetes. Till now no scientific research works have been carried out on this poly herbal formulation, the author is interested in validating the efficacy of *Elathy urundai* as a potent anti-diabetic drug through physico chemical analysis, phytochemical analysis, biochemical analysis, pharmacological studies.

In Siddha system, *Kaya Kalpa* medicines which can revive the beta cells secreting insulin have been explained. This is a metabolic disorder, hence *Kaya Kalpa* medicines which can revive the ruined beta cells have been prescribed by the Siddhars long ago.

Universal blue circle symbol for diabetes

AIM AND OBJECTIVES

2. AIM AND OBJECTIVES

Aim

Diabetes mellitus is a metabolic disorder and now it is a lifestyle disorder which is a great threat to the mankind. As there is a great change in our day to day activities, food habits, physical activities etc., this disease has got its own spurt worldwide. Particularly India is the leading one as it is getting more westernized. This disease needs a prolonged treatment with safe medication and quality living. This could be achieved by Siddha system of medicine as it not only treats the disease but also brings the overall wellbeing of human.

According to the Siddha literature ***Elathy Urundai*** was used for Diabetes mellitus. Thus the aim of this study was to validate the safety and efficacy of the test drug ***Elathy Urundai*** for Anti-Diabetic Activity in Streptozotocin induced Wistar albino rats.

Objectives

The following methodology was adopted to evaluate the safety and efficacy of the test drug in this study

- Collection of various Siddha and modern literature relevant to the study.
- Preparation of the drug according to the classical Siddha literature.
- Physicochemical and phytochemical investigation of the test drug.
- Evaluate bio-chemical analysis of the test drug to derive acidic and basic radicals.
- To estimate the percent of elements, functional groups and particle size through instrumental analysis of the trial drug.
- Evaluation of the Acute and 28 days repeated dose Toxicity of test drug according to OECD guidelines.
- Evaluation of pharmacological study of the drug through the following activities
    - Anti-Diabetic Activity-Streptozotocin induced diabetes in Wistar albino rats.
    - Anti-Dyslipidemic Activity-Triton WR 1339 induced Dyslipidemic in Wistar albino rats.
    - Anti-Oxidant Activity-Through DPPH assay
- To scrutinize all the above studies to establish the potency of ***Elathy Urundai.***

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

3.1. DRUG REVIEW

3.1.1. GUNAPADAM ASPECT

Ingredients of *Elathy Urundai*

- *Elam (Elettaria cardamomum)*
- *Kirambu (Syzygium aromaticum)*
- *Ilavanga pathiri (Cinnamomum tamala)*
- *Aavarai panchangam (Cassia auriculata)*
- *Seenthir kizhangu (Tinospora cardifolia)*
- *Thannervittan kizhangu (Asparagus recemosus)*
- *Thamarai valaiyam (Nelumbo nucifera)*
- Butter Milk

1.Elam[8A]***:***

Alternative names: *Aanje, Korangam, Thudi*

Vernacular names:

Malayalam : Elattari

Sanskrit : Ela

Kan : Elakki

Properties:

Suvai (Taste) : Kaarppu

Thanmai (Nature) : Veppam

Pirivu (Division) : Kaarppu

Actions :

- Stomachic
- Carminative
- Stimulant

General character:

"தொண்டை வாய்கவுள் தாலுகு தங்களில்
தோன்றும் நோயதி சாரம்பன் மேகத்தால்
உண்டை போல்எழுங் கட்டி கிரிச்சரம்
உழலை வாந்தி சிலந்தி விஷஞ்சுரம்
பண்டை வெக்கை விதாகநோய் காசமும்
பாழுஙஞ் சோமப் பிணிவிந்து நட்டமும்
அண்டை யீளைவன் பித்தம் இவைக்கெல்லாம்
ஆல மாங்கமழ் ஏல மருந்ததே…

- தேரன் குணவாகடம்

It is used in the treatment of mouth and palate disorders, cough, diarrhea, poisonous stings of insect bites and increases the sperm count.

2. Kirambu[8B]**:**

Alternative names: *Anjukam, Urkadam, Karuvai kirambu, Sossam, Therali, Varangam*

Vernacular names:

Malayalam: Kirampu

Sanskrit : Lavanga

Kan : Lavanga

Properties

Suvai (Taste) : Kaaram

Thanmai (Nature) : Veppam

Pirivu (Division) :Kaarppu

Action:

- Stimulant
- Carminative
- Stomachic

General characters:

"பித்த மயக்கம் பேதியொடு வாந்தியும்போம்
சுத்தவிரத் தக்கடுப்புந் தோன்றுமோ-மெத்த
இலவங்கங் கொண்டவருக் கேற் சுகமாகும்
மலமங்கே கட்டுமென வாழ்த்து.
சுக்கிலநட் டங்கர்ண சூர்வியங்க லாஞ்சனந்தாட்
சிக்கல்விடாச் சர்வா சியப்பிணியு-மக்கிக்குட்
டங்கப் பூவோடு தரிபடருந் தோன்றிலில்
வங்கப்பூ வோடுரைத்து வா.

- அகத்தியர் குணவாகடம்

It is used in the treatment of giddiness, vomiting, dysentery, chronic diarrhea, ear diseases, scabies and eye diseases.

3. *Ilavanga pathiri*[8C]:

Alternative names : *Thalisapattiri, Thamalapattiri*

Vernacular names

Malayalam - Paccila

Sans - Tamalapatram

kan - Kadu lavanga patte

Properties:

Suvai (taste) - *Kaarpu*

Thanamai (nature) - *Veppam*

Pirivu (division) - *Kaarpu*

Actions:

- Stimulant
- Carminative
- Stomachic
- Diaphoretic

General characters:

"மேகசுரம் சீதசுரம் வெட்டைசுவா சங்காசம்
தாகபித்தம் வாந்திசர் வாசியநோய் -மேகத்தின்
கட்டியோடு தாதுநட்டங் கைப்பருசி போக்கிவிடும்
இட்டஇல வங்கத் திலை".

- அகத்தியர் குணவாகடம்

Uses

It is used in the treatment of fever, asthma, cough, thirst, vomiting, mouth ulcers, abscess, oligospermia and helps in modulating the bitter taste.

4. *Aavarai panchangam*[8D]

Alternative names*: Aavari, Aaverai, Eamaputpi, Makare, Aaguli, Thalapodam*

Vernacular names:

Malayalam : Avara

Sans : Telapotakam

Kan : Tangadi-gida,Avare-gida

Properties:

Suvai (Taste) : Thuvarppu

Thanmai (Nature) : Thatpam

Pirivu (division) : Inipu

Action:

- Astringent
- Tonic
- Refrigerant
- Alterative

General characters:

"மோகத்தி னாலே விளைந்தசலம் வெட்டையனல்

ஆகத்தின் புண்ணோ டருங்கிராணி-போகத்தான்

ஆவாரைப் பஞ்சகங்கொள் அத்திசுரம் தாகமும்போம்

ஏவாரைக் கண்மடமா தே"

- அகத்தியர் குணவாகடம்.

It is used in the treatment of diabetes, leucorrhea, body heat, ulcers, amoebic dysentery, fever and thirst.

5. *Seenthir kizhangu*[8E]

Other names: *Amirthavalli, Somavalli, Amirthai, Amirthakodi, Gundali*

Vernacular name*:*

Malayalam : Amrutha

Sans : Guduchi

Kan : Amruta-valli

Properties

Suvai (Taste) *: Kaippu*

Thanmai (Nature) *: Veppam*

Pirivu (Division) *: Kaarppu*

Actions:

- Alterative
- Antiperiodic
- Aphrodisiac
- Demulcent
- Stimulant
- Stomachic

General character:

"சீந்திற் கிழங்கருந்தத் தீபனமாம் மேகவகை

போந்தவுதி ரப்பித்தம் பொங்குசுர-மாந்தம்

அதிசாரம் வெய்யகணம் ஆம்பலநோ யோடே

கதிவிடமுங் கெட்டுவிடுங் காண்"

- அகத்தியர் குணவாகடம்

It is used in the treatment of diabetes, hypertension, fever, dysentery, tuberculosis, cures poisonous bites and increases appetite.

6. *Thannervittan kizhangu*[8F]:

Other names: *Thaneervittan, Sathaveli, Sathavari, Sathamoolam, Sathavari, Neervali, Narayani, Neervitan, Aagerugam, Varivari, Uthagamoolam, Seekuvai, Paranai, Peruthanthi.*

Vernacular name:

Malayalam : Sataveri

Sans : Shatavari

kan : Satmula

Properties:

Suvai (taste) : Inippu

Thanamai (nature) : Thatpam

Pirivu (division) : Inippu

Actions:

- Nutritive
- Demulcent
- Galactagogue
- Aphrodisiac
- Antispasmodic

General character :

"நீரிழிவைப் போக்கும் நெடுநாட்சு ரத்தையெலாம்
ஊரைவிடுத் தோடவு ரைக்குங்காண்-நாரியரே! ஆ
வெண்ணீர்பெய் சோமநோய் வெட்டை யனல்தணிக்குந்
தண்ணீர்விட் டான்கிழங்கு தான்"

- அகத்தியர் குணவாகடம்

It is used in the treatment of diabetes, chronic fever, osteomyelitis, intense body heat.

7. *Thamarai valaiyam*[8G]:

Alternative names: *Aravindham, Ellimanai, Suriya natpu, Ponmanai, Vindham, Pundareegam, Padhumam, Kamalam, Nalinam, Mundagam, Amberugam, Salasam, Pangerugam.*

Vernacular name:

Malayalam : Aravindam

Sans : Pankaja

kan : Tavare

Properties:

Suvai *(taste)* : Kaarpu

Thanamai (*nature)* : Veppam

Pirivu *(division)* : Kaarpu

Actions:

- Stimulant
- Carminative
- Stomachic
- Diaphoretic

General character :

"பருத்தநற் றாமரைப்பூ பல்வாந்தி நோயைத்
துரத்திவிடும் இன்னுஞ் சொல்வோ- கரத்தில்
எடுத்தணைக்கக் கண்குளிரும் ஏகுஞ் சுரமும்
எடுத்தவி தாகமும்போம் எண்"

- அகத்தியர் குணவாகடம்

It is used in the treatment of eye irritation, fever, thirst.

8. Butter milk[9]**:**

"வீக்க மகோதரமுள் வீறுகுன்மம் பாண்டுபித்தந்
தாக்குமருந் திட்டததி சாரமொடு-கூக்குரலே
மாறாத் திரிதோஷ மந்தமனற் றாகம்போம்
வீறாவின் மோருக்கு மெய்"

- பதார்த்தகுண சிந்தாமணி.

It is used in the treatment of swelling, ascites, stomach ache, anaemia, diarrhea, balances *tridhoshas,* cures indigestion and thirst.

3.1.2. BOTANICAL ASPECT

1. Elettaria cardamomum[10A]

Taxonomical Classification

Kingdom : Plantae

Class : Monocotyledons

Serious : Epigynaes

Family : Zingiberaceae

Genus : Elettaria

Species : cardamomum

Distribution : Cultivated throughout India.

Description : Tall herbaceous perennial with branching stock, 1.5-5 m in height,

Parts used : Seeds

Chemical constituents:

Moton seeds contains trace waxes, alpha-terpinylacetate, cineole, linalyl acetate, limonene and linalool, limonene, cineole terpinolene, myrcene.[11]

Properties and uses:

The seeds are aromatic, acrid, cooling, stimulant, carminative, digestive, stomachic, diuretic, cardio tonic. It is used in treatment of asthma, bronchitis, cardiac disorders, burning sensation, hyperdipsia.

2. Syzygium aromaticum[12A]***:***

Classification

Kingdom : Plantae

Class : Dicotylendons

Order : Myrtales

Family : Myrtaceae

Genus : Syzygium

Species : aromaticum

Distribution : Cultivated in south India.

Description : A pyramidal evergreen tree usually up to 12m in height with a single main stem bearing oblique branches. Leaves simple. Flowers are highly aromatic.

Parts used : Dried flower buds.

Chemical constituents**:** The main constituents of the essential oil are phenylpropanoids such as carvacrol,thymol,eugenol and cinnamaldehyde.[13]

Properties and uses:

The cloves are acrid, bitter, aromatic, refrigerant, carminative, stomachic, anti-spasmodic, emollient, anthelmintic, sialagogue, rejuvenating. It is used in the treatment of anorexia, cough, burning sensation, skin diseases, helminthiasis, impurity of breast milk, neuralgia, dental carries and tuberculosis.

3. **Cinnamomum tamala**[10B]

Classification

Kingdom : Plantae

Class : Dicotyledons

Order : Laurales

Family : Lauraceae

Genus : Cinnamomum

Species : tamala

Distribution : Distributed around Himalayas, in areas of 900-2400m elevation

Description : A moderate sized evergreen tree 7.5m in height with dark brown or blackish rough bark. Leaves are simple opposite, alternate. Flowers pale yellowish in axillary and terminal.

Part used : Leaves

Chemical constituents:

Cinnacassiol A, B and C, trans-cinnamic acid,cinnamaldehyde and eugenol[14].

Properties and uses:

The leaves are bitter, sweet, aromatic, thermogenic, anthelmintic, diuretic, stimulant, carminative and tonic.

4. ***Cassia auriculata***[10C]

Classification

Kingdom : Plantae

Class : Dicotylendons

Order : Rosales

Family : Casesalpiniaceae

Genus : Cassia

Species : auriculata

Distribution : Throughout central and south India.

Description : A much branched shrub with reddish brown braches, leaves with subulate glands, cordate stipules at their bases. Flowers are bright yellow in sub-terminal axillary corymbs.

Parts used: Root, bark, leaves, flowers, seeds.

Chemical constituents:

Flowers –β-Sitosterol And Kaempferol.

Leaves - β-Sitosterol And Emodin.

Pod Husk -β-Sitosterol, Chrysophanol, Emodin, Ruβicidin and Nonacosan-6-One [15].

Properties and uses:

Astringent, alterative, anthelmintic, aphrodisiac and stomachic. It is used in the treatment of diabetes, skin diseases, leprosy, ulcers, tumors, asthma, chyluria, chronic purulent conjunctivitis.

5. ***Tinospora cordifolia***[12b]

Classification

Kingdom : Plantae

Class : Dicotylendons

Order : Ranales

Family : Menispermaceae

Genus : Tinospora

Species : cordifolia

Distribution : Throughout India in forests.

Description

A large extensively spreading glabrous, perennial deciduous twinner with succulent stem and papery bark. Leaves are simple, alternate. Fruits are drupe, red when ripe.

Parts used: Stem

Chemical constituents:

Tinosporine, Tinosporon, Tinosporic Acid, Tinosporol, Tinosporide, Tinosporidine, Columbin, Palmar, Berberine, Giloin, B-Sitosterol, Cordifolide, Unosporin, Cordifol, Cordifolon[16].

Properties and uses:

Astringent, anti-periodic, anti-spasmodic, anti-inflammatory, anti-emetic, carminative, aphrodisiac, rejuvenating. It is used in the treatment of hyperdipsia, helminthiasis, dyspepsia, chronic fever, inflammation, gout, vomiting, skin disease, leprosy, anemia.

6. ***Asparagus racemosus***[17]***:***

Classification:

Kingdom : Plant kingdom

Class : Dicotyledons

Order : Coronarieae

Family : Liliaceae

Genus : Asparagus

Species : racemosus

Distribution : Cultivated throughout India at an elevation upto 1300m.

Description:

An armed, climbing undershrub with woody stem, young stems very delicate, brittle and smooth, and leaves reduced to minute chaffy scales and spines cladodes triquetrous. Flowers are white; the tuberous succulent roots are 30 cm to a meter or more in length, fascicled at the stem base, smooth, tapering at both ends.

Parts used: Tuberous roots

Chemical constituents:

4-Saponins - Shatavarin I, II, III, IV[18]

Shatavarin IV is a Glycoside Of Sarsapogenin With 2 Moles Of Rhamnose And 1 Mol of Glucose [19].

Flowers And Fruits - Glucosides Of Quercetin, Eutin,Hyperoside.

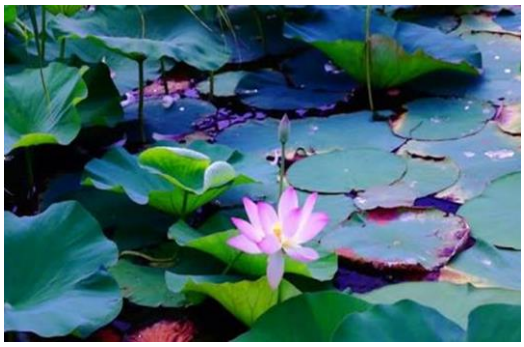
Properties and uses:

Emollient, nervine tonic, galactogogue, anodyne, aphrodisiac, diuretic, rejuvenating.

It is used to treat nervous disorder, dyspepsia, diarrhea, gonorrhea, leucorrhea, hyperdipsia, burning sensation.

7. ***Nelumbo nucifera***[20]***:***

Classification:



Kingdom : Plantae

Class : Dicotyledons

Order : Ranales

Family : Nymphaeaceae

Genus : Nelumbo

Species : nucifera

Distribution : Throughout India in marshes and ponds, upto an elevation of 1,800 m.

Description

Large handsome aquatic herb with slendar, enlongate branched creeping, rhizomes, sending out roots at the nodes.

Leaves are peltate, 60-90 cm or more in diameter, petioles very long, smooth or with small or rosy with a centrally located yellow obconical spongy torus in which carpels are sunken, fruits ovoid, nut- like achenes.

Parts used : Whole plant

Chemical constituents:

Megastigmane,nelumnucifoside A and new eudesmane sesquiterpene,eight alkanoids and eleven flavoinoids[21]

Properties and uses:

Astringent, emollient, diuretic, antipyretic, cardio tonic, anthelmintic. It is used to treat nervous exhaustions, skin diseases, pectoralgia, spermatorrhea, leucoderma, leprosy, haemorrhoids, cholera, fever, hepatopathy.

3.2. REVIEW OF THE DISEASE

3.2.1. Siddha aspect of the disease

Madhumegam

Madhumegam comes under *Neerinai Perukkal Noi* as mentioned in *Therayar* Maha *Karisal.*

நீரிருவினைக் குணத்தை நீயறிவிரித்து சொல்வாம்

நீரினைப் பெருக்கலொன்று நீரினையருக்க லொன்று

நீரிழிவுடனே கொல்லும் நீர்க்கட்டு வினைகளென்று

நீணிலமுறைக் குமிந்த நீர்நிறைக் குணத்தைக் கேளாய்

\- தேரையர் மகாகரிசல்

Synonym

Mega neer, Vegu moothiram, Innipu neer, Neerizhivu, Thithippu neer, Pramegham.

Definition[22]

Madhumegam is a clinical condition characterized by frequent urination resulting in deterioration and diminution of the seven thathus and loss of weight.

Abdomen distention, slurring of speech, peripheral neuritis, lassitude, dyspnoea are the symptoms of *Madhumegam*.

Etiology

- Diet habits
- Sexual indulgence
- obesity
- Psychosomatic cause
- Hereditary
- Excess stimulation of *moolatharam*

General symptoms

1. Thirst
2. Polydipsia
3. Anorexia
4. Delirium
5. Sleeplessness
6. Hiccough
7. Anaemia
8. Polyuria
9. Cough
10. Dyspnea
11. Pain in the hip and burning sensation
12. Loss of Weight

Classification of the disease

Classification of *Megam* [23]

Vali - 4	***Iyam - 10***
1. Neimananeer	*1.Iyaneer*
2. Pasumananeer	*2.Thuimaineer*
3. Seezhmananeer	*3.Moolaineer*
4. Sadhaimananeer	*4.Ilaneer*
Azhal - 6	*5.Ulneer*
1. Yanaikozhupu Mananeer	*6.Thavalaneer*
2. Katrazhai Mananeer	*7.Kazhuneer*
3. Chunna Mananeer	*8.Thenneer*
4. Ennipu Megam	*9.Uppuneer*
5. Palingu Neer	*10.Kavichuneer*
6. Muyal Kuruthi Neer	

Diabetes mellitus is a clinical entity in a modern medicine is closely resembles one of the types of "*Pitha Premeham*" i.e "*Madhumegam"*.

Clinical features

Polyuria, polyphagia, polydipsia, perspiration, exhaustion, insomnia, giddiness and loss of weight even at normal consumption of food.

Pathophysiology [24]

In the disease *Madhumegam*, due to internal and external causes affect balance in the ratio of *Vali*, *Azhal*, and *Iyam*. The imbalance affects the *Keelnokkukal*, which in turn affect the seven *Udal thathukkal*. *Saram* gets affected and there is loss of appetite. *Seiner* also get affected with the net result even if the patient eats more nourished food (Polyphagia), there won't be any improvement in health.

An imbalance in *Iyam* does imply an imbalance in other two *kutrams* too and causes derangement of *dasavayu* and seven *Udal thathukkal* which causes the disease and other complications.

3.2.2. MODERN ASPECT OF THE DISEASE

Diabetes mellitus

Diabetes mellitus is one of the most common endocrine disorders. It is a clinical syndrome characterized by hyperglycaemia with or without glycosuria, resulting from an absolute or relative deficiency of insulin, affecting carbohydrate, protein and fat metabolism. It may be due to impairment of insulin production or its release by Beta cells of islets of Langerhans. Long standing metabolic derangement is associated with functional and structural changes in many organs especially the vascular system leading to Diabetic retinopathy, neuropathy and nephropathy and atherosclerosis.

The three major types of Diabetes mellitus

- Type 1 Diabetes.
- Type 2 Diabetes.
- Gestational Diabetes mellitus

Type 1 diabetes mellitus

It is called as the insulin dependent, immune mediated or juvenile onset diabetes. People with this type of diabetes produce very little or no insulin. They need injections of insulin every day to control the glucose level in the blood.

Type II diabetes

It is called as the non-insulin dependent diabetes. It is also known as late-onset diabetes and it is characterized by insulin resistance and relative insulin deficiency. Though the disease is highly genetic in origin some risk factors such as excess weight, inactivity, high blood pressure and poor diet plays a major role for its development.

Gestational Diabetes mellitus (GDM)

It is the presence of high glucose level in the blood during pregnancy. It usually disappears after the pregnancy but the women and her children are at high risk of developing type II diabetes later in their life.

Complications [25]

Virtually every tissue and organ is biochemically and structurally altered as a consequence of the hyperglycemia of diabetes and result in complications.

Two biochemical mechanisms appear to be involved in the development of many complications.

(i) Non enzymatic glycosylation

It is the process by which glucose attaches to amino group of proteins without the aid of enzymes. That can cause structural and functional abnormalities of the involved proteins to form Advanced Glycation End Products (AGE), which cause microangiopathy, nephropathy. The concentration of Glycosylated Hemoglobin (HbA_1C) in the blood is now used clinically as a measure of therapeutic control.

(ii) Intracellular Hyperglycemia with disturbances in Polyol pathways

The second biochemical mechanism operates in the aorta, lens of the eye, kidney and peripheral nerves. These tissues are endowed with an enzyme, aldoses reductase that facilitates the accumulation of sorbital and fructose in cells of the hyperglycemic patient. As a result of the intracellular accumulation of sorbital and fructose an osmotic gradient is established and excessive amounts of water enter the cells from the extra cellular compartment. The cells then swell and are damaged and contribute to neuropathy and cataracts.

1. Diabetic ketoacidosis

It is a major medical emergency and serious cause of mortality principally in people with type 1DM. It has a mortality rate of 6-10%.In newly diagnosed patients of type 1 DM there is failure of endogenous insulin and in NIDDM it is due to inadequate exogenous insulin or a stressful conditions like myocardial infarction, hyperthyroidism, pheochromocytoma, trauma, pregnancy, drugs like cocaine. In these conditions there is increase in production of counter regulatory hormones like epinephrine, cortisol, glucagon and growth hormone.

The cardinal biochemical features are

1.hyperglycaemia

2.hyperketonaemia

3.metabolic acidosis

Clinical features [26]

(i) Polyuria, thirst, weight loss, weakness, nausea, vomiting, leg cramps, blurred vision and abdominal pain which is common in children

(ii) The striking features are those of salt and water depletion, with loss of skin tone, furred tongue, cracked lips, tachycardia, hypotension and reduced intra ocular pressure.

(iii) Cold extremities, tachycardia, air hunger (Kussmaul breathing), acetone odour in breath.

(iv) Hypothermia, confusion, drowsiness and coma.

2. Hypoglycaemia:

It is the fall of blood glucose levels below 50-60mg/dl at which level symptoms occur in normal persons. Development of symptoms depends on the prevailing blood glucose levels and individual susceptibility.

Symptoms of hypoglycaemia

- Autonomic symptoms like palpitation, sweating, tremors, anxiety.
- Neuroglycopenic symptoms: tiredness, dizziness, drowsiness, difficult to concentrate and dysphasia.
- Lethargy, coma, convulsions
- Permanent brain damage.
- Sudden death due to cardiac arrhythmias (‘dead in bed’ syndrome).

3. Cardiovascular system

- Macroangiopathy
- Microangiopathy

4. Ocular complication

- Diabetic Cataract
- Diabetic Retinopathy
- Glaucoma develops in 6% cases.

5. Renal complications

- Renal arteriosclerosis.
- Pyelo nephritis - This is very common and may lead to chronic renal failure.
- Micro albuminuria
- Papillitis, Necroticans- The renal papillae will show necrosis, ultimately leading to viaemia.
- Kimmelstiel – Wilson syndrome (K.W. syndrome)
- This is develops in average 10 years duration clinically patient will present feature of nephritic syndrome.

6. Diabetic neuropathy

This is an early and common complication affecting 39% of diabetic patients. Metabolic neuropathy develops due to hyperglycaemia and this subsides with proper control of Diabetes.

- Peripheral neuritis (30%)
- Autonomic imbalance
- Diabetic amyotrophy
- Charcot's joint

7. Sexual and Genital Complications

Impotence and frigidity may develop erectile dysfunction in males is particularly important. Balanitis and Balanoposthitis are common complications in males. These are due to secondary infection as urine contains heavy amount of sugar and nitrogenous materials. Leucorrhoea may develop in females.

8. Pulmonary complications

Tuberculosis is very common in diabetes other infective complications like pneumonia, bronchopneumonia, pleurisy etc.

9. Effect on pregnancy and neonates

There may be miscarriages and abortions, toxaemias of pregnancy, hydramios etc Herculian child may be born of diabetic mothers due to secretion of excess of growth hormone.

10. Diabetic Foot

The foot is a frequent site for complications in patients with diabetes, so foot care is particularly important. Tissue necrosis is common reason for hospital admission in diabetics may end in amputation.

Treatment

Anti-Diabetic drugs

Insulin [27]

1. Insulin was first isolated from the Pancreas in 1922 by Banting and Best. Insulin is a polypeptide containing two chains of amino acids linked by disulfide bridges having molecular weight of 5808.

2. It is synthesized in the endoplasmic reticulum of the β cells as Preproinsulin that give rise to Proinsulin which undergoes peptic cleavage to form Insulin and C-peptide. C-peptide connects α and β chains.

3.The half-life of insulin in the circulation in human is about 5 minutes and cleared from circulation within 10-15 minutes and stored in beta cell granules and discharged into interstitial fluid under appropriate stimulus, enters portal circulation and liver traps 50-60% and remaining enter peripheral circulation. It is degraded by enzyme Insulinase in liver, in the kidneys and muscles.

Preparations of insulin

Insulin preparation differs in their source and duration of action. Based on the ource they may be classified as bovine, porcine and human insulin.

Table No.1. Insulin types

Conventional insulin	
▪ Short and rapid acting ▪ Intermediate acting ▪ Long acting	Regular Semilente Lente Isophane insulin(NPH) Ultralente Protamine zinc insulin
Highly purified insulins	
▪ Single peak insulin ▪ Monocomponent insulins	Regular Lente Regular Lente
Human insulin	Regular Lente Ultralente Isophane
Insulin analogs ▪ Rapid acting ▪ Long acting	 Insulin lispro Insulin aspart Insulin glargine Insulin detemir
Insulin mixtures	Combinations of 20-50-% regular with 80-50% NPH (Neutral Protamine Hagedorn) insulins.

Figure no.1. Insulin

Insulin and its doses [28]

Regular/plane insulin	-	SOLUBLE INSULIN 40 IU/ml
LENTE INSULIN, ISOPHANE INSULIN, PROTAMINE ZINC INSULIN	-	40 IU/ml inj for sub-cutaneous use.
Highly purified regular	-	ACTRAPID MC-40,100 IU/ml inj
Highly purified lente Iu/ml	-	LENTARD, MONOTARD MC 40
Highly purified NPH	-	INSULATARD-40/ml
Mixture of regular + NPH insulin	-	MIXTARD 40IU/ml
Human actrapid	-	regular 40,100 IU/ml inj
Human monotard	-	Lente
Human insulatard	-	NPH
Human mixtard	-	Regular NPH
Humalog100U/ml inj 100 IU/ml (30ml vial)	-	NOVALOG 100 IU/ml inj, FLEXPAN

Oral Anti-Diabetic Drugs [29]

The main disadvantage of insulin is the need for injection. The advance of oral hypoglycaemics came as a boon to millions of NIDDM patients with early and mild diabetes. Sulfonylureas were available in 1950s. We now have 5 groups of oral hypoglycaemics

- sulfonylureas,
- biguanides,
- meglitinides,
- alpha glucosidase inhibitors
- Thiazolidinediones.

Table No.2. Mechanism of action and adverse effects of oral antidiabetic drug

Mechanism of action and adverse effects of oral antidiabetics		
Oral antidiabetics	**Major mechanism**	**Adverse effects**
Sulfonylureas	↑ insulin release from pancreas ↑ tissue sensitivity to insulin	Hypoglycaemia Cholestatic jaundice Disulfiram like reaction
Biguanides	↓ hepatic gluconeogenesis ↑ tissue sensitivity insulin	Diarrhea, metallic taste, rarely lactic acidosis
Meglitinides	↑ insulin release from pancreas	Hypoglycaemia
Thiazolidinediones	↑ glucose transport into tissues ↓ hepatic gluconeogenesis	Weight gain, oedema, may precipitate CCF, risk of hepato toxicity
α glucosidase inhibitors	↓ glucose absorption ↓ hydrolysis of disaccharides	Flatulence, diarrhoea, abdominal distension

Status of anti-diabetic drugs

For the patients with IDDM, insulin is the only treatment as there is insulin deficiency due to destruction of β cells. Sulphonylureas need functional β cells for their action and therefore are not useful in IDDM. Uncomplicated NIDDM patients not controlled by diet and exercise are given oral hypoglycaemics. Mild NIDDM patients with recent onset diabetes, age above 40 years at onset of diabetes, obese with fasting sugar less than 200 mg/dl are candidates for oral hypoglycaemics.

Some of the other Siddha drugs used for diabetes mellitus

- *Navalkottai Chooranam*
- *Seenthil Chooranam*
- *Sirukurinchan Chooranam*
- *Kompupagal Chooranam*

- *Thiripala Chooranam*
- *Navathanya Chooranam*
- *Santhiraganthi Chooranam*
- *Maruthampattai kashayam*
- *Atthiyathi kashayam*
- *Aanandhabairava Mathirai*
- *Mahalinga Mathirai*
- *Sanjeevi kirutham*
- *Meghaadi Kuligai*
- *Karpoorathi Mathirai*
- *Abragha parpam*
- *Naga parpam*
- *Velvanga parpam*
- *Kaandha sendooram*

3.3 PHARMACOLOGICAL REVIEW

Pharmacological study of Anti-Diabetic activity in animal models

Models for insulin dependent diabetes

1. Chemically induced diabetes

It is the most commonly used animal model of the diabetes mellitus. It is classified into three categories that include agents that

- Specifically damage the β-cell
- Cause temporary inhibition of insulin production
- Decrease the efficacy of insulin in target tissues
- In general chemicals in the first category are of interest as they reproduce lesions resembling IDDM[30].

2. Alloxan – induced diabetes

Alloxan is a cyclic urea drug analog and it was reported to produce permanent diabetes in animals.

Mechanism of action

The mechanism by which it induces diabetes is not very clear. Alloxan is highly reactive molecule that is readily reduced to diuleric acid, which is then auto oxidized back to alloxan resulting in the production of free radicals. These free radicals damage the DNA of the β cells and cause cell death. Second mechanism proposed for alloxan is its ability to react with protein SH group, especially the membrane proteins like glucokinase on the β cells; finally resulting in cell necrosis however, there are major species differences in response to alloxan[31].

Procedure

Animal models		**Dose**
Rabbits (2 to 3 kg)	–	150 mg /kg
Wistar Rats (150-200gm)	_	100-175mg/kg
Male beagle dogs (15-20kg)	–	60mg/kg
Monkeys	–	65-200mg/kg

All the animals which are administered with alloxan receive glucose and regular insulin for one week and food ad libitum. Then single daily dose of 28 IU insulin is administered. There is a triphasic change in the blood glucose level. At first there is a hyperglycemia at 2 hrs, then hypoglycemic phase at 8 hrs and finally an increase at 24 hrs.

3. Streptozotocin (STZ) - induced diabetes

STZ (2-deoxy-2-(3-methyl-3-nitrosourea) 1-D-glucopyranose) is a broad spectrum antibiotic produced from the Streptomyces achromogens. The Diabetogenic activity of STZ was first described by Rakieten[32].

Mechanism of causing β cell damage

- By process of methylation
- Free radical generation
- Nitric oxide production

Procedure

STZ induces diabetes in all species of animals.

Animal model		**Dose**
Rats	-	50-60mg/kg
Mice	-	175-200mg/kg
Dogs	-	15mg/kg

After administration of the STZ the blood glucose level shows the triphasic change. Hyperglycemia at 1 hr, followed by hypoglycemia for 6hrs, and there is a stable hyperglycemia by 24-48 hrs.

4. Hormone induced diabetes

Dexamethasone, a long acting glucocorticoid is used to produce diabetes. Dexamethasone is administered to the rats in the dose of 2-5 mg/kg twice daily over a number of days.

5. Diabetes induced by the viral agents

Viruses are thought to be one of the etiologic agents for IDDM. Viruses may produce Diabetes mellitus by

- Infecting and destroying of β-cells in pancreas.
- A less infecting or cytologic variant producing a comparable damage by eliciting immune auto reactivity to the β-cells.
- Viruses producing systemic effect, not directly affecting β-cells.

Various numbers of viruses are used to induce diabetes. They are RN picorno virus, coxsackie-B4 (CB4), mengo – 2T, encephalomylocarditis (emc-d and m variants), double stranded RNA viruses, reovirus and lymphocytic choriomeningitis virus (LMCV). Primary isolates of these human pathogenic agents are generally not pancreatotrophic or ilytic to mouse b-cells and must be adapted for growth either by inoculation into suckling mice or by passage in cultured mouse β-cells[33] .

6. Surgically induced diabetes

Surgical removal of all or parts of the pancreas induce diabetes; in partial pancreatectomy more than 90% of the organ is removed to produce diabetes. Depending on the amount of intact pancreatic cells, diabetes may range in duration from a few days to several months. Total removal of the pancreas results in an insulin dependent form of diabetes, and insulin therapy is required to maintain experimental animals. The portion of the pancreas usually left intact following a subtotal pancreatic resection is typically the anterior lobe or a portion thereof[34].

The use of pancreatectomy in combination with chemical agents, such as alloxan and STZ, produces a stable form of Diabetes mellitus in animals, such as cats and dogs, that does not occur when each procedure is applied an dependently. The combination therapy reduces the organ damage associated with chemical induction and minimizes the interventions, such as enzyme supplementation, necessary to maintain a pancreatectomized animal.

7. Insulin antibodies-induced diabetes

Giving bovine insulin along with CFA to guinea pigs produces anti-insulin antibodies. Intravenous injection of 0.25-1.0 ml guinea pig anti-insulin serum to rats induces a dose dependent increase in blood glucose levels up to 300 mg%. This unique effect to guinea pig anti-insulin serum is due to neutralization of endogenous insulin by the insulin antibodies. It persists as long as the antibodies are capable of reacting with insulin remaining in the circulation. Slow i.v. infusion or i.p. injection prolongs the effect for more than a few hours. However, large doses and prolonged administration are accompanied by ketonemia, ketonuria, glycosuria and acidosis and are fatal to the animals. After lower doses, the diabetic syndrome is reversible after a few hours[35].

3.4. PHARMACEUTICAL REVIEW

Pharmaceutics is a discipline of pharmacy that deals with the process of turning a new chemical entity to be used safely and effectively by the patients. (Formulation of pure drug substance into dosage form)

Siddha pharmaceutics has very minute chemical processes in it. It has several chemical processes like purification of raw substances, grinding them with herbal juices for several days and subjecting the ground material to fire by way of *putam* process. Medicines prepared according to the above methods undergo several chemical changes.

Siddha medicines are classified into internal medicines (32) and external medicines (32). The drug taken for dissertation is in the form of *Mathirai.* Other names of *Mathirai* are *Kuligai, Urundai, and Vattam. Mathirai* comes under the category of internal medicines.

Purification of the drugs included

Purification of the drugs is mainly done to remove the toxicities, impurities like soil, dust, clay present in the drugs. Also the drugs when subjected to heat like roasting or soaked in liquids undergo certain chemical reactions such as oxidation of toxic substances to non-toxic, reduction of some poisonous chemicals to non-poisonous ones or undergo enzymatic reactions. In these ways, not only the toxicities and impurities are removed but also enhanced the potency of the drugs.

Concept and Terminology of pills

It is a pill prepared from a finely ground paste of drugs. The term *Mathirai* is the most fitting category of medicines as besides indicating the form of medicine that is pills. It also means that the minimal dosage unit is one pill (*Mathirai* means 1 unit). Preparation of *Mathirai* includes various processes like, extraction of juices, making decoction, preparing powders, grinding pastes and rolling into pills or pressing into tablets. The raw drugs are dried in the sun or shade and the drugs which are aromatic are to be roasted separately. The raw drugs are purified and grounded separately, then the compounded drug be grounded in a mortar for the prescribed period with the addition of prescribed juices and decoctions.

If green drugs are to be added they should be made into fine paste before being used. Vegetable drugs which require frying are fried and powdered. However, scented herbal drugs like cinnamon leaves and cloves, cinnamon bark are dried only in shade as otherwise their volatile oil are lost by drying.

The individual drugs should be separately weighed after being powdered and then taken in the prescribed ratio. After the pill mass has been prepared by following, the processes outlined in the recipe, it is convenient to roll it into long uniform pencils and then cut into bits of uniform length to give suitable pill weight and then rolling a pill from each piece. This is a fast process to prepare uniform pills as pinching and rolling every time is invariably a tiresome, tedious and time consuming messy process. The pill mass when rolled between the fingers should not stick. This is the correct consistency for rolling into pills. The pills should be always dried in a warm, dry shady place and never under the sun because volatile matters in the pills are easily lost and photochemical breakdown of active principles are faster in sunlight of the tropics. If the pill mass sticks to the fingers, a speck of ghee may be smeared on the fingers. Pills should be well dried in shade [36].

Storage and Usage

Almost all the *Mathirai* contain highly active ingredients. Hence they should be preserved in well stopper glass vials with relevant labels and instructions. If the *Mathirai* lose their natural shape, colours, smell, taste etc, it is not advisable to consume them. If properly stored, we can keep them for a year.

Preparation of *Mathirai* in Manufacturing Units

In the manufacturing unit, *Chooranam* is compressed into tablets. Tablets are unit forms of solid medicinal substances with or without suitable diluents prepared by compressing and they are mostly discoid in form. Binders like Gum acacia, lubricants like liquid paraffin and disintegrators like Talcum powder are used. *Chooranam* is first prepared according to the above procedures. Then the ingredients are mixed with in the form of granules before compressing as tablets. Too much fine powder refuses to form satisfactory tablets and so they must be mixed with some adhesive substances or binders such as gum acacia. To prevent the sticking of the tablets to the punches and dyes a lubricant like liquid paraffin is added. If the tablet is to dissolve quickly, a disintegrator like talcum powder is added [37].

Shelf life of the drugs

The shelf life of the drugs depends on the effectiveness of the preparation. The efficacy, smell, taste and appearance of the drugs gradually change as time goes on resulting in reduced potency thereby the desired effect is not attained. But some drugs appear to be good externally in spite of reduced efficacy. So they should not be considered for consumption and should be discarded. The shelf life of *Mathirai* is 1 year. According to recently published guidelines by Ayush, the shelf life period of *Mathirai* is 2 years. Also the following are the analytical parameters of specifications of *Mathirai*[38] .

Table No.3. Testing parameters for *Mathirai*-AYUSH guidelines [39]

S.No	Tests
1	Description, Colour, Odour
2	Weight Variation
3	Disintegration Time (Not more than 15 minutes)
4	Identification TLC/ HPTLC/GLC
5	Assay
6	Test for heavy/toxic metals Mercury Arsenic Cadmium Lead
7	Microbial Contamination Total Bacterial count Total Fungal count
8	Test for specific pathogen E.coli Salmonella species Pseudomonas aeruginosa Streptococcus aureus
10	Test for aflatoxins B1, B2, G1, G2

Traditional tests for *Mathirai*

Characters:

- Non sticky on rolling.
- No cracks over the surface after drying.
- Shall be rolled uniformly over the plane surface.

Based on these characters the drug is assessed as the appropriate one for medication.

3.5. LATERAL RESEARCH

***Cassia Auriculata* (Anti-bacterial and Anti-oxidant activity)**

The ethanol, methanol and aqueous extracts of dry and fresh flower possess Anti-Bacerial activity and also shows the presence of phytochemicals such as terpinoids, tanins, flavonoids, saponin, cardiac glycosides, and steroids were observed.

The Anti-Oxidant activity of ethanoland methanol extract of Cassia auriculata flowers were studied in two assays based on the decolorization of the radical monocation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. It showed anti-oxidant activity in both the assays [40].

***Syzygium Aromaticum* (Antibacterial activity and Hepatoprotetive activity)**

Methanolic extract of clove showed maximum zone of inhibition 24mm against S. aureus while minimum was 19 mm against P. aeuginos. Ethanolic extract of clove showed maximum zone of inhibition 20 mm against P. aeuginosa while minimum was 18mm against E. coli.

50 % ethanolic extract of S. aromaticum can attenuate liver necrosis in animal model. Liver injury was induced by single intraperitoneal injection of thioacetamide (400 mg/kg b. w.). Oral administration of the extract (800 mg/kg b. w.) for three consecutive days could significantly ameliorate the changes associated with hepatic injury, particularly the levels serum biochemical markers of liver injury and

oxidative stress. The protective action may be attributed to eugenol, which has been shown to be present in the test extract [41].

Elettaria Cardamomum

Anti-oxidant and anti-microbial activities of essential oil and various oleoresins of *Elettaria cardamomum* (seeds and pods)

Elettaria cardamomam compares the effect of the different extraction solvent used (chloroform.methonol, ethanol and diethyl ether) on the Anti-oxidant, Anti-microbial activities of essential oils and oleoresins [42].

***Cinnamomum tamala* (Anti-Bacterial activity)**

Cinnamomum tamala and their chemical constituents were investigated in this study [43]

***Tinospora Cardifolia* (Anti-ulcer and anti-oxidant activity)**

Tinospora cordifolia was tested for its anti-ulcer and anti-oxidant activity in rats, pepticare studied on gastric secretion and gastric ulcers in pylorus-ligation and gastric ulcers in pylorus-ligation and ethanol-induced gastric mucosal injury in rats[44]

Nelumbo Nucifera

Cardioprotective effect of *Nelumbo nucifera* flower extract Agaistisoproterinol induced oxidative stress in male albino Swiss rats

The present study investigate the cardioprotective effect of Nelumbo nucifera flower in isoproterinol induced rats. The positive hypertrophy response of isoproterinol caused a severe oxidative stress in the myocardium through increased lipid peroxidation. Nelumbo nucifera was administered intraperitonealy at a dose of 200mg/kg for a period of 30days [45].

***Asparagus racemosus*(Hypolipidemic and anti oxidant activity)**

It is reducing the cholesterol levels and as an anti-oxidant in hypercholesteremic rats.[46]

MATERIALS AND METHODS

4. MATERIALS AND METHODS

Preparation of the Drug

Selection of drug

The trial drug "***Elathy Urundai*"** was prepared as per Siddha literature **"*Sarabenthirar vaithiya muraigal.*"**

Ingredients

Elam *(Elettaria cardamomum*)	- 1 *Palam (*35gm)
Kirambu (*Syzygium aromaticum)*	- 1 *Palam* (35gm)
Ilavangap pathiri (*Cinnamomum tamala)*	- 1 *Palam* (35gm)
Aavarai panchangam (*Cassia auriculata*)	- 1 *Palam* (35gm)
Seenthir kizhangu (*Tinospora cardiofolia*)	- 1 *Palam(*35gm)
Thannervittan kizhangu (*Asparagus racemosus*)	- 1 Palam(35gm)
Thamarai valaiyam (*Nelumbo nucifera*)	- 1Palam (35gm)
Butter milk	- 50ml

Collection of the drugs

The drugs such as *Elam, Kirambu, Ilavangappathiri, Aavarai panchangam, Seenthir kizhangu, Thamaraivalaiyam* were procured from Ramasamy chettiyar Raw Drug Stores at Parry's corner, Chennai.

Butter milk from Departmental store.

Ref No:GSMC/PGGM/061-067/2014-2017.

Identification and Authentication

All the raw drugs were identified and authenticated by the Botanist and experts of Gunapadam Department (Pharmacology) at Government Siddha Medical College, Arumbakkam, Chennai 106.

The specimen samples of the identified raw drugs were preserved in the laboratory of P.G Gunapadam for future references.

Method of purification

Elam

All the impurities like sand and dust were removed, then slightly roasted and powdered.

Kirambu

All the impurities like sand and dust and the flower buds were removed, then slightly roasted and powdered.

Ilavangappathiri

Pale coloured and infected leaves were removed, dried and powdered.

Aavarai panchangam

All the impurities like sand and dust were removed, then slightly roasted and powdered.

Seenthirkizhangu

Soaked in water, skin and the veins were removed then dried and powdered.

Thannervittankizhangu

Soaked in water, skin and the veins were removed then dried and powdered.

Thamaraivalaiyam

All the impurities like sand and dust were removed, then slightly roasted and powdered.

Method of preparation

The Purified drugs were ground separately to powder and powder was sieved through a white cloth (vasthrakayam) to get the fine Choornam then the Choornam

was taken in the stone mortar and ground with butter milk to get a paste. Make tablets from the paste and allow it to dry.

Preservation

The medicine was preserved in a clean, air tight container.

Administration of the drug

Form of the medicine	: Pills
Route of administration	: Enteral
Dose	: 2 pills
Time of administration	: 2 times a day
Indication	: Diabetes and Polyuria.

Ingredients of *Elathy Urundai*

Elettaria cardamomum

Figure No.1

Syzygium aromaticum

Figure No.2

Standardisation of the drug

World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care. The process of evaluating the quality and purity of herbal drugs by means of various parameters like physical, chemical and biological observation is called standardization. Standardization of the this drug comes under the following categories

- Physio-chemical analysis
- Phyto chemical analysis
- Bio chemical analysis

Organoleptic evaluation

The Organoleptic characters of the sample were evaluated which include evaluation of the formulation by its colour, odour, size etc.

Colour examination

Ten tablets were taken into watch glasses and positioned against white back ground in white tube light. Its colour was observed by naked eye and results are noted.

Odour examination

Ten numbers of tablets were smelled individually. The time interval among two smelling was kept two minutes to overturn the effect of previous smelling. Odour of *Elathy Urundai* was noted in results table.

Size examination

The diameter of ten tablets was measured by Vernier caliper. The mean value of diameter was noted.

Physio-Chemical investigation

Physico-chemical studies like total ash, water insoluble ash, acid Insoluble ash, loss on drying at 105°C and pH were done at, Central Research Institute, Chennai.

Solubility Test

A pinch of the sample (*Elathy Urundai*) was taken in a dry test tube and shaken well with distilled water. A little amount of the sample (*Elathy Urundai*) is shaken well with con. HCl and then Con. H_2SO4. Solubility was observed.

Determination of Total Ash

About 2 g of the ground drug (*Elathy Urundai*) was accurately weighed in a silica dish and incinerated at a temperature not exceeding 450º until it was free from carbon, cooled and weighed. The percentage of ash with reference to the air-dried drug was calculated.

Determination of Water Soluble Ash

Total ash was heated up to 600^0C with 25 ml of distilled water for 10 minutes and the residue was ignited in the furnace to get a constant weight. And the weight was calculated.

Determination of Acid Insoluble Ash

The ash obtained was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and insoluble matter was collected in an ash-less filter paper, washed with hot water and put up in flames to constant weight. The percentage of acid-insoluble ash with reference to the air dried drug was noted.

Determination of Moisture Content (Loss on Drying)

This procedure was done to determine the amount of volatile matter in the drug. A sample of 10 gram of the drug (*Elathy Urundai*) was placed in a tarred evaporating dish after accurately weighting without preliminary drying. The dish was dried at a temperature of 105^0C for about 5 hours and again weighed. The drying and weighing procedure was repeated again and again until the difference between two successive weights was not more than 0.25%. And the weight was calculated.

pH value

Potentiometrically pH value was determined by a glass electrode and a pH meter. The pH of the *Elathy Urundai* was written in results column.

Tablet Disintegration test

Each *Elathy Urundai* was placed in each of the six tubes of the basket present in the disintegration apparatus. The apparatus was operated by using water as the immersion fluid maintained at 35-39 °C. At the end of 30 min, the basket is lifted from the fluid and the state of the tablet is observed. The disintegration time of *Elathy Urundai* was recorded[47].

Weight variation test

It was carried out to make sure that, each number of tablets contains the proper amount of drug. The test was carried out by weighing the 20 tablets individually using analytical balance, then the average weight was calculated and comparing the individual tablet weights to the average[48].

The percentage of weight variation is calculated by using this formula.

$$\% \text{ of wt. variation} = \frac{\text{Individual wt} - \text{Average wt}}{\text{Average wt.}} \times 100$$

Table No. 4. Weight variation limits of Tablets (IP)

Average weight of tablets	Maximum percentage of weight difference allowed
80 mg or less	±10.0
Between 80 mg and 250 mg	±7.5
250 mg and more	±5.0

Phytochemical analysis

The Phytochemical screening of the extract gives general idea regarding the nature of chemical constituents present in the crude drug. The phytochemical tests were done as the method illustrated in[49].

Test for Alkaloids

A small portion of solvent free extracts was stirred separately with few drops of dilute hydrochloric acid and filtered & tested carefully with various alkaloidal reagents.

Mayer's reagent - Cream precipitate

Dragendroff's reagent - Orange brown precipitate

Hager's reagent - Yellow precipitate

Wagner's reagent - Reddish brown precipitate

Test for Carbohydrates and Reducing Sugars

The minimum amount of extracts was dissolved in 5ml of distilled water & filtered. The filtrate was subjected to test for carbohydrates & glycosides.

a) Molisch's test

The filtrate 1 ml was treated with 2-3 drops of 1% alcoholic alpha naphthol& 2ml concentrated sulphuric acid was added along the sides of test tube. Violet ring was observed at the junction of 2 layers which showed the presence of carbohydrate.

b) Benedict's test

The filtrate 1 ml was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) Fehling's test

The filtrate 1 ml was treated with equal volume of Fehling's solution A and B and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Test for Glycosides

The extract was hydrolyzed with dil. HCl and subjected to test for glycosides.

a) Modified Borntrager's test

To the hydrolysate extract, 1 ml of Ferric chloride solution was added and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in the ammoniacal layer indicates the presence of Anthranol glycosides.

b) Legal's test

The hydrolysate extract was treated with Sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of Cardiac glycosides.

Test for Saponins

The extract 0.5 ml was shaken with 5 ml distilled water. The presence of saponins was indicated by formation of copious lather.

Test for Tannins

Gelatin test

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Test for Phenolic compounds

To 0.5 ml of extract, 1 ml of alcoholic ferric chloride solution was added. Formation of bluish green or bluish black indicates the presence of Phenolic compounds.

Test for Phytosterol

Ferric chloride – acetic acid test

1 ml of extract was treated with 1 ml of chloroform and then, 2 ml of ferric chloride acetic acid reagent was added followed by 1 ml of conc. sulphuric acid. Appearance of reddish pink colour shows the presence of phytosterol.

Test for Diterpenes

Copper acetate test

1 ml of extract was dissolved in water and treated with 3-4 drops of Copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Test for Triterpenes

Salkowski's test

1 ml of extract was treated with 1 ml of chloroform followed by 1 ml of conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour shows the presence of triterpenes.

Test for Flavonoids

a) Alkaline reagent test

To 1 ml of extract, 1 ml of 10% sodium hydroxide solution was added. Formation of dark yellow colour indicates the presence of flavonoids.

b) Lead acetate test

To 1 ml of extract, 3-4 drops of 10% lead acetate solution was added. Formation of yellow precipitate indicates the presence of flavonoids.

c) Ferric chloride test

To 1 ml of extract, 3-4 drops of ferric chloride solution was added. Formation of dark green colour indicates the presence of flavonoids.

d) Shinoda test

To 1 ml of extract, few mg of magnesium turnings was added followed by few drops of conc. hydrochloric acid and boiled for five minutes in a boiling water bath. Formation of red colour indicates the presence of flavonoids.

Test for Proteins and Free Amino Acids

a) Xanthoproteic test

To 1 ml of extract, 3-4 drops of conc. nitric acid was added. Formation of yellow precipitate indicates the presence of proteins.

b) Million's test

To 0.5 ml of extract, 2.5 ml of Million's reagent was added. Formation of white precipitate and the precipitate warmed indicates the presence of proteins.

c) Biuret test

To 0.5 ml of extract, 2.5 ml of diluted Biuret reagent was added. Appearance of purple colour or brick red precipitate showed the presence of proteins and free amino acids.

Test for Quinones

Sodium hydroxide test: To 0.5 ml of extract, 1 ml of 10% sodium hydroxide was added. Appearance of blue or green or red colour shows the presence of quinones.

TLC/ HPTLC finger print studies

HPTLC finger printing was carried out as per the reference[50].

Preparation of spray reagent-vanillin-sulphuric acid reagent

Vanillin (1g) was dissolved in ice cold ethanol (95ml). Add to 5ml of cooled concentrated sulphuric acid. Ice was added and stirred well. The solution was stored in refrigerator.

Chromatographic conditions

Instrument : CAMAG (Switzerland).

Sample applicator : Camag Linomat - IV applicator with N_2 gas flow.

Photo documentation System : Digi store - 2 documentation system with Win Cat and video scan software.

Scanner : Camag HPTLC scanner - 3 (030618), Win Cats - IV.

Development Chamber : Camag HPTLC 10X10, 10 X 20 twin trough linear development chamber.

Quantity applied : 5, 10 µl for extracts and 5 µl for standards

Stationary phase : Aluminium plate pre-coated with silica gel 60 (E. Merck)

Plate thickness : 0.2 mm.

Mobile Phase : Chloroform extract - Toluene: Ethyl acetate (9:2).

Scanning wavelength : 254 nm

Laboratory condition : 26 ± 5^{o}C and 53 % relative humidity

The plate was developed up to a height of 8 cm, air dried, spots were observed under the UV light at 254 and 366 nm. Finally the plates were derivatized using vanillin-sulphuric acid reagent heated at 105° till colour spots appeared.

Bio-Chemical analysis

The bio-chemical analysis was done to identify the acid and basic radicals present in the *Elathy Urundai*.

Preparation of extract

5g of *Elathy Urundai* was taken in a 250 ml clean beaker and 50 ml of distilled water was added, boiled well and allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water.

Preliminary basic and acidic radical studies

Test for basic radicals

1. Test for Potassium

To a pinch of the *Elathy Urundai* 2 ml of Sodium nitrate and 2 ml of Cobalt nitrate solution in 30% glacial acetic acid was added and observed for the presence of yellow precipitate.

2. Test for Calcium

To 2 ml of *Elathy Urundai* extract, 2 ml of 4% Ammonium oxide solution was added and observed for the formation of white precipitate**.**

3. Test for Magnesium

To 2ml of *Elathy Urundai* extract, drops of Sodium hydroxide solution was added and watched for the appearance of white precipitate.

4. Test for Ammonium

To 2ml of *Elathy Urundai* extract few ml of Nessler's reagent and excess of Sodium hydroxide solution are added for the appearance of brown colour.

5. Test for Sodium

Hydrochloric acid was added with a pinch of the *Elathy Urundai,* made as paste and introduced into the blue flame of Bunsen burner and observed for the appearance of intense yellow colour.

6. Test for Iron (Ferrous)

The *Elathy Urundai* extract was treated with Conc. HNO_3 and Ammonium thiocyanate and waited for the appearance of blood red colour**.**

7. Test for Zinc

To 2 ml of the *Elathy Urundai* extract drops of Sodium hydroxide solution was added and observed for white precipitate formation.

8. Test for Aluminium

To the 2m1 of the *Elathy Urundai* extract sodium hydroxide was added in drops and changes are noted.

9. Test for Lead

To 2 ml of *Elathy Urundai* extract 2ml of Potassium iodide solution was added and noted for yellow coloured precipitate**.**

10. Test for Copper

a. A pinch of *Elathy Urundai* was made into a paste with con. HCl in a watch glass and introduced into the non-luminous part of the flame and noted for blue colour appearance.

b. To 2 ml of *Elathy Urundai* extract excess of Ammonia solution was added and observed for the appearance of blue coloured precipitate**.**

11. Test for Mercury

To 2m1 of the *Elathy Urundai* extract sodium hydroxide solution was added and noted for yellow precipitate formation**.**

12. Test for Arsenic

To 2 ml of the *Elathy Urundai* extract 2ml of Sodium hydroxide solution was added and observed for brown or red precipitate and noted.

Test for acid radicals

1. Test for Sulphate

To 2 ml of the *Elathy Urundai* extract 5% of Barium chloride solution was added and observed for the appearance of white precipitate.

2. Test for Chloride

The *Elathy Urundai* extract was treated with Silver nitrate solution and observed for the appearance of white precipitate.

3. Test for Phosphate

The *Elathy Urundai* extract was treated with Ammonium molybdate and conc. HNO_3 and observed for the appearance of yellow precipitate.

4. Test for Carbonate

The *Elathy Urundai* extract was treated with conc.HCl and observed for the appearance of effervescence.

5. Test for Fluoride & Oxalate

To 2ml of *Elathy Urundai* extract 2ml of dil.acetic acid and 2ml calcium chloride solution was added and heated and watched for cloudy appearance.

6. Test for Nitrate

To 1 gm of the *Elathy Urundai*, Copper turnings was added and again conc.H_2SO_4 was added, heated and the test tube was tilted vertically down and observed for any changes.

Microbial load

Availability of bacterial load

Enumeration of bacteria by plate count – agar plating technique

The plate count technique is one of the most routinely used procedures because of the enumeration of viable cells by this method[51].

Principle

This method is based on the principle that when a material containing bacterium is cultured, every viable bacterium develops into a visible colony on a nutrient agar medium. The numbers of colonies therefore are the same as the number of organisms contained in the *Elathy Urundai.*

Dilution

A small measured volume of *Elathy Urundai* is mixed with a large volume of sterile water called the diluent or dilution blank. Dilution is usually made in multiples of ten. A single dilution is calculated as follows:

$$\text{Dilution} = \frac{\text{Volume of the sample}}{\text{Total volume of the sample and the diluents}}$$

Requirements

- Sample or Bacterial suspension
- 9 ml dilution blanks (7)
- Sterile petri dishes (12)
- Sterile 1 ml pipettes(7)
- Nutrient agar medium (200 ml)
- Colony counter

Procedure

1. Label the dilution blanks as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}.
2. Prepare the initial dilution by adding 1 ml of the *Elathy Urundai* extract into a 9 ml dilution blank labelled 10^{-1} thus diluting the sample 10 times.
3. Mix the contents by rolling the tube back and forth between hands to obtain uniform distribution of organisms.
4. From the first dilution transfer 1 ml of the suspension while in motion, to the dilution blank 10^{-2} with a sterile and fresh 1 ml pipette diluting the original specimen to 100 times.
5. From the 10^{-2} suspension, transfer 1 ml of suspension to 10^{-3} dilution blank with a fresh sterile pipette, thus diluting the original sample to 1000 times.
6. Repeat this procedure till the sample has been diluted 10,000,000 times, every time using a fresh sterile pipette.
7. From the appropriate dilutions transfer 1ml of suspension while in motion, with the respective pipettes, to sterile petri dishes. Three petri dishes are to be used for each dilution.

8. Approximately 15 ml of the nutrient medium is added, melted and cooled to 45^0c, to each petri dish containing the diluted *Elathy Urundai* extract. Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.
9. Allow the plates to solidify.
10. Incubate these plates in an inverted position for 24-48 hours at 37^0c.

Observation

Observe all the plates for the appearance of bacterial colonies. Count the number of colonies in the plates.

Calculate the number of bacteria per ml of the original suspension as follows:

$$\text{Organisms per millimetre} = \frac{\text{Number of colonies (average of 3 repleates)}}{\text{Amount plated} \times \text{dilution}}$$

Sophisicated instrumental analysis

FT-IR (Fourier Transform Infra-Red)

Model	:	Spectrum one: FT-IR Spectrometer
Scan Range	:	MIR 450-4000 cm-1
Resolution	:	1.0 cm-1
Sample required	:	50 mg, solid or liquid.

It was the preferred method of infrared spectroscopy. FT-IR was an important and more advanced technique. It was used to identify the functional group, to determine the quality and consistency of the sample material and can determine the amount of compounds present in the sample. It was an excellent tool for quantitative analysis [52].

FT-IR was the most advanced and the major advantage was its

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated.

In FT-IR infrared was passed from a source through a sample (*Elathy Urundai*). This infrared was absorbed by the sample (*Elathy Urundai*) according to the chemical properties and some are transmitted. The spectrum that appears denotes the molecular absorption and transmission. It forms the molecular fingerprint of the *Elathy Urundai.* Like the finger print there was no two unique molecular structures producing the same infrared spectrum. It was recorded as the wavelength and the peaks seen in the spectrum indicates the amount of material present [53].

SEM (Scanning Electron Microscope)

In scanning electron microscope high-energy electron beam was focused through a probe towards the sample (*Elathy Urundai).* Variety of signals was produced on interaction with the surface of the sample (*Elathy Urundai*). This results in the emission of electrons or photons and it was collected by an appropriate detector [54].

The types of signal produced by a scanning electron microscope include

- Secondary electrons
- back scattered electrons
- characteristic x-rays, light
- specimen current
- Transmitted electrons.

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample [55].

ICP-OES (Inductively Coupled Plasma Optic Emission Spectrometry)

Manufacturer: Perkin Elmer

Model: Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer (ICP)

Principle

An aqueous sample was converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which was a high temperature zone

(8,000– 10,000ºC). The analysts are heated (excited) to different (atomic and/or ionic) states and produce characteristic optical emissions (lights). These releases are separated based on their respective wavelengths and their strengths are measured (spectrometry). The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification was an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions. With respect to other kinds of analysis where chemical speciation was relevant (such as the concentration of Ferrous Iron or Ferric Iron), only total essential concentration was analyzed by ICP-OES [56].

Application

The analysis of major and minor elements in solution *Elathy Urundai.*

Objectives

- Determine elemental concentrations of different metals.
- Learn principles and operation of the ICP-OES instrument.
- Develop and put on a method for the ICP-OES sample analysis.
- Enhance the instrumental conditions for the analysis of different elements probes the outer electronic structure of atoms.

Mechanism

In plasma emission spectroscopy (OES), a *Elathy Urundai* solution was presented into the core of inductively coupled argon plasma (ICP), which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. This light was collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light was then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardization values[57].

The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was done in SAIF, IIT MADRAS, and Chennai-36 using Perkin Elmer Optima 5300 DV.

Sample preparation

Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby *Elathy Urundai* are introduced in liquid form for analysis.

100 mg *Elathy Urundai* was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowed for few minutes untill the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution.

The digested *Elathy Urundai* solution was shifted into plastic containers and labeled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106.

FTIR (Fourier Transform Infrared Spectroscopy)

Fig 2.1: FTIR Instument

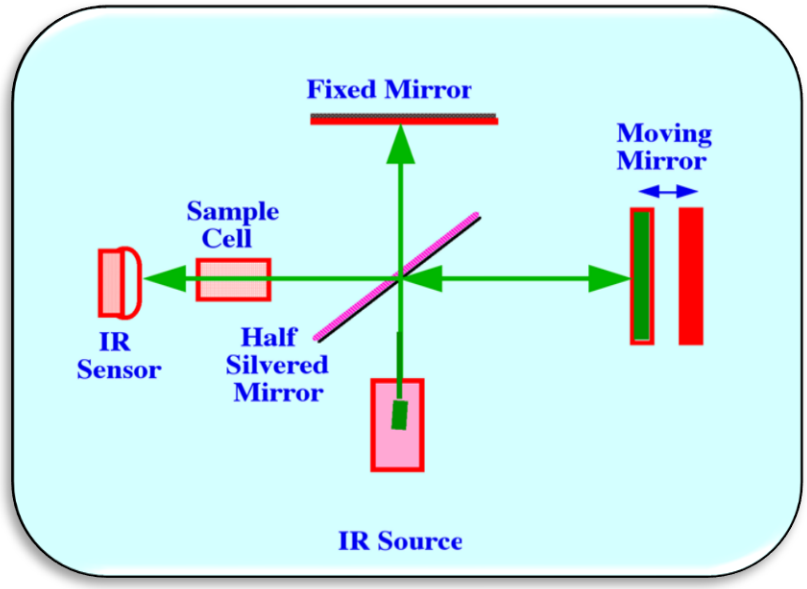


Fig 2.2: FTIR Mechanism

SEM – Scanning Electron Microscope

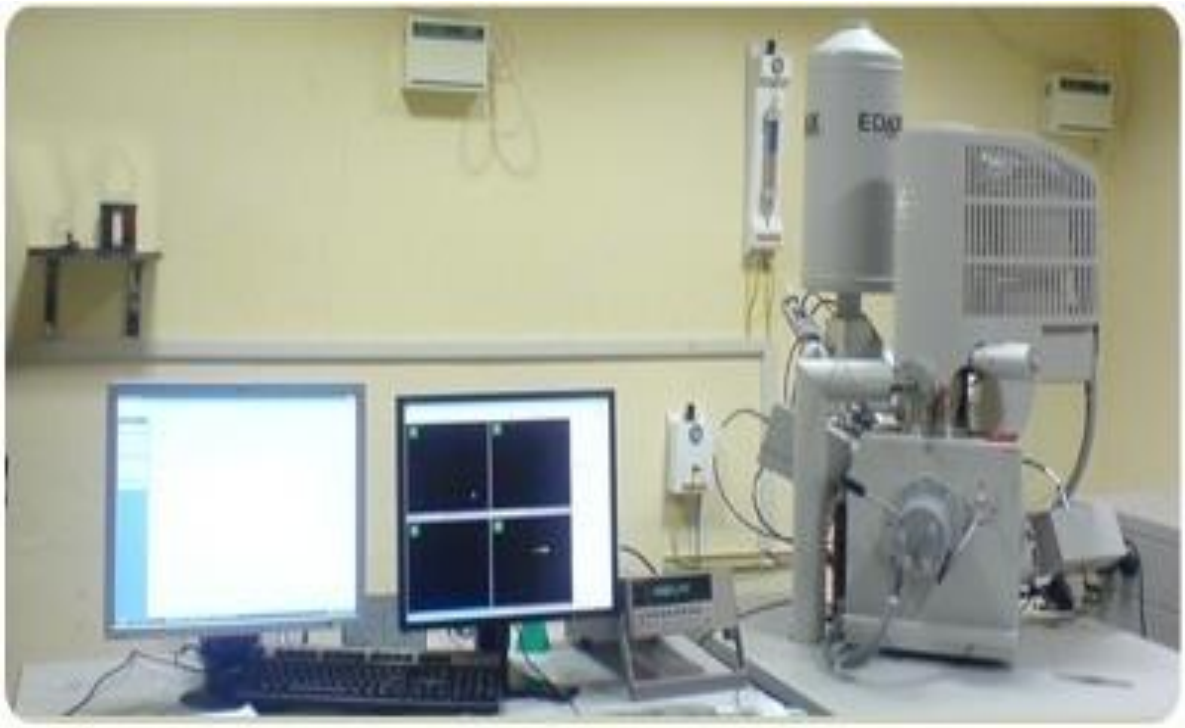


Fig 2.3: SEM Instument

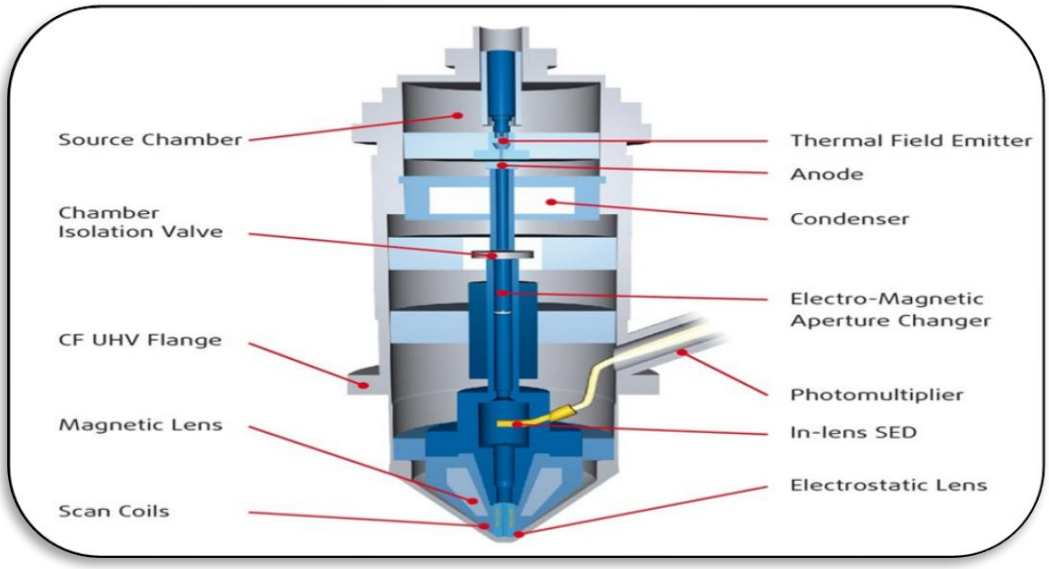


Fig 2.4: SEM Mechanism

IC-POES (Inductively Coupled Plasma Optic Emission Spectrometry)

Fig 2.5: ICP-OES Analyser (Perkin Elmer Optima 5300 DV)

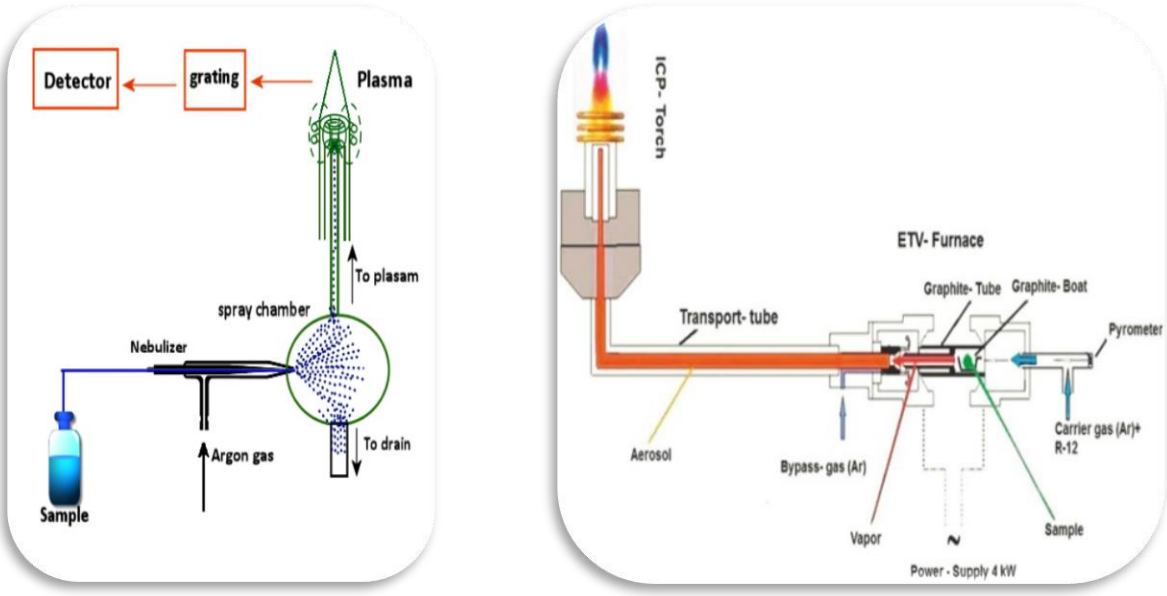


Fig 2.6: Mechanism of ICP-OES analyser

TOXICOLOGICAL STUDIES

Acute oral toxicity – OECD Guidlines – 423

Introduction

The acute toxic class method was a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. Morbid animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed and are considered in the interpretation of the test results in the same way as animals that died on test. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co – operation and Development, Guideline-423. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) under CPCSEA (Approval no: IAEC/XLIV/26/CLBMCP/2014) at C.L.Baid Metha College of pharmacy, Thuraipakkam, Chennai.

Animal: Healthy wistar albino female rat weighing 200–220 gm.

Principle

It was the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, information was obtained on the acute toxicity of the test substance to enable its classification. The substance was administered orally to a group of experimental animals at one of the defined doses. The substance was tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; − no further testing was needed – dosing of three additional animals with the same dose – dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes [58].

Studied carried out at three female rats under fasting condition, signs of toxicity was observed for every one hour for first 24 hours and every day for about 14 days from the beginning of the study.

Methodology

Selection of animal species

The preferred rodent species was rat, although other rodent species may be used. Healthy young adult animals of commonly used laboratory strain Swiss albino rat were obtained from Animal house of King Institute, Guindy, Chennai. Females should be nulliparous and non-pregnant. Each animal at the commencement of its dosing should be between 8 and 12 weeks old and its weight should fall in an interval within ±20 % of the mean weight of the animals. The studies were conducted in the animal house of C.L.Baid Metha College of pharmacy, Thuraipakkam, Chennai.

Housing and feeding conditions

The temperature in the experimental animal room should be 22ºC (+3ºC). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hrs dark. For feeding, conventional laboratory diets may be used with an unlimited supply of

drinking water. Animals may be grouped and tagged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions.

Experiment procedure

Administration of doses

Elathy Urundai was prepared as per the classical Siddha literature was suspended in 2% CMC with uniform mixing and was administered to the groups of Wistar albino rats. It was given in a single oral dose by gavages using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration.

The visual observations included skin changes, mobility, aggressiveness, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 hours prior to the administration of the test suspension. Finally, the number of survivors were noted after 24 hours and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Number of animals and dose levels

Since this *Elathy Urundai* has been under practice for long time and likely to be non-toxic, a limit test at one dose level of 2000 mg/kg body weight will be carried out with 6 animals (3 animals per step).

Duration of Study : 48 hours

Evaluation : 14 Days

Limit test

The limit test was primarily used in situations where the experimenter has information indicating that the test material was likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

Observations

- The animals were observed individually after dosing at least once during the first 30mins and periodically during the first 24 hours.
- Special attention: First 1-4 hours after administration of drug.
- It was observed daily thereafter for a total of 14 days, except when they needed to be removed from the study and killed humanely for animal welfare reasons or are found dead.

a. Mortality

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hours following drug administration on day 1 of the experiment and daily twice thereafter for 14 days.

b. Body weight

Body weights will be recorded at Day 1, 2, 7 and 14 of the study

c. Cage-side observation

These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavioral patterns. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

d. Gross necropsy

All animals (including those which die during the test period are removed from the study) will be subjected to gross necropsy. Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and

abdominal cavities and their contents, brain, eye, thymus, lungs, heart, spleen, liver, kidneys, adrenals, testes and uterus of all animals.

Histopathology

Microscopic examination will be carried out in organs to show the evidence of any toxicity in gross pathology.

Data and reporting

All the data were summarised in tabular form showing the animals used, number of animals displaying signs of toxicity, the number animals found dead during the test or killed for humane reasons, a description and the time course of toxic effects and reversibility of necroscopic findings.

Test substance and Vehicle

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing *Elathy Urundai* with 2% CMC solution and it was found suitable for dose accuracy.

Justification for choice of vehicle

The vehicle selected as per the standard guideline was pharmacologically inert and easy to employ for new drug development and evaluation technique[59].

Repeated dose 28 days oral toxicity of *Elathy Urundai* on rats– (OECD-407 guidelines)

Justification for Dose Selection

The results of acute toxicity studies in Wistar albino rats indicated that *Elathy Urundai* was non-toxic and no behavioral changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected for the study were100mg/kg, 200 mg/kg and 400 mg/kg body weight. The oral route was selected for use because oral route was considered to be a proposed therapeutic route [60].

Preparation and administration of dose

Elathy Urundai at three doses respectively was suspended in 2 ml of 2% CMC in distilled water. It was administered to animals at the dose levels of 100, 200 and 400 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

Methodology

Randomization, Numbering and Grouping of Animals

Ten rats (Five Male and Five Female) were in each group randomly divided into four groups for dosing up to 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

Observations

Experimental animals were kept under observation throughout the course of study for the following

Body Weight

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

Clinical signs

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality

All animals were observed twice daily for mortality during entire course of study.

Functional Observations

At the end of the 4th week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' were assessed.

Laboratory Investigations

Following laboratory investigations were carried out on day 29 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes. On 28th day of the experiment, 24 hours urine samples were collected by placing the animals in the metabolic cage with free access to tap water but no feed was given.

The urine was free from fecal contamination. Toluene was used as a preservative while collecting the sample. The sediments present in the urine were removed by centrifugation and the collected urine was used for biochemical estimations. On 29th day, the animals were fasted for approximately 18 hours, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Haematological Investigations

Blood samples of control and experimental rats was analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

Biochemical Investigations

Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate

aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Urine analysis

Urine samples were collected on end of treatment for estimation of normal parameters. The estimations were performed using appropriate methodology.

Necropsy

All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, spleen, brain, heart, and lungs were recorded. The relative organ weight of each animal was then calculated as follows;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of animal on sacrifice day (g)}} \times 100$$

Histopathology

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 400 mg/kg were preserved and were fixed in 10% formalin for 24 hours and washed in running water for 24 hours. Samples were dehydrated in an auto technique and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin. The organs included heart, kidneys, liver, ovary, pancreas, brain, spleen and stomach, of the animals were preserved they were subjected to Histopathological examination.

Statistical analysis

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by Dunnet's multi comparision test using a computer software programme GRAPH PAD INSTAT-3 version.

PHARMACOLOGICAL ACTIVITY

Anti-Diabetic activity of *Elathy Urundai*

Screening the drug *Elathy Urundai* against Streptozotocin (STZ) induced Diabetes in Wistar albino Rats

Experimental Animals

The animals were divided into 5 groups each constituting 6 rats. Group I were normal rats, Group II were STZ (55 mg/kg b.w., i.p) induced diabetic rats. Group III STZ (55 mg/kg b.w., i.p) induced diabetic rats were treated with Glibenclamide 5mg/kg b.w/p.o Group IV STZ (55 mg/kg b.w., i.p) induced diabetic rats were treated with *Elathy Urundai* 200mg/kg b.w/ p.o Group V STZ (55 mg/kg b.w., i.p) induced diabetic rats were treated with *Elathy Urundai* 400mg/kg b w/p.o for 28 days.

Methodology

Induction of Diabetes

Diabetes was induced in male Wistar albino rats aged 2–3 months (180–200 g body weight) by intra peritoneal administration of STZ (single dose of 55 mg/kg b.w) dissolved in freshly prepared 0.01 M citrate buffer, pH 4.5.

After injection the animals had food and water *ad libitum* and were given 5% glucose in their drinking water for the first 24 hours to counter any initial hypoglycemia. The development of diabetes was confirmed after 72 hours of the Streptozotocin injection. After 72 hours of STZ injection under mild anesthesia the blood was withdrawn from the tip of the tail of each rat and the blood glucose level was analyzed. Animals with more than 250 mg/dl was considered as diabetic.

Fasting blood glucose levels was measured before the administration of extracts. The blood glucose levels were checked on 0th, 7th, 14th, and 21st day of the treatment period. Blood was collected from snipping of the rat tail. Blood glucose levels were measured [61].

Experimental Design

Diabetic rats were divided into three groups with six animals in each group.

Table No.5. Experimental design

Groups	Treatment
Group I	Normal Control
Group II	Diabetic control- STZ (55 mg/kg)
Group III	Diabetic control- Glibenclamide (5 mg/kg)
Group IV	Diabetic control- *Elathy Urundai* 100mg/kg
Group V	Diabetic control- *Elathy Urundai* 200mg/kg

Blood collection

All the experimental rats were fasted overnight and the blood was withdrawn through puncturing retro orbital sinus on the 5^{th} day, 15^{th} day and 20^{th} day of post induction period to determine blood glucose level by GOD-POD kit method. The change in body weight was observed throughout the treatment period in experimental animals [62].

Statistical Analysis

All the values were expressed as Mean ± S.D. The differences between control and treatment groups were tested for significance using ANOVA followed by Dunnet's t test. $P<0.05$ were considered significant.

Anti-Dyslipidemic activity

Triton WR 1339 induced Dyslipidemia for *Elathy Urundai*

Experimental protocol

Total number of groups	: 4
Number of animals / group	: 6
Sex	: Both
Strain	: Wistar albino rats
Body Weight	: 200 – 250 g

Surfactant administration

Surfactant	: 10% Triton WR – 1339
Route of administration	: Intravenously
Vehicle	: Saline

Test drug administration

Vehicle	: 2% CMC
Route of administration	: Oral
Drug dose	: *Elathy Urundai* - 200 mg/kg b.wt

Procedure

Wistar rats weighing 200–350 g were starved for 18 hours and then injected intravenously with 10% Triton WR 1339 (isooctyl-polyoxyethylene phenol).

Phase I: Serum cholesterol levels increase sharply 2–3 times after 24 hours.

Phase II: The hypercholesterolemia decreases nearly to control levels within the next 24 hours.

The test drug (*Elathy Urundai*) employed or the solvent for the controls are administered simultaneously with the Triton injection or 22 hours thereafter. Serum cholesterol analyses are made 6, 24, and 48 hours after Triton injection.

Mechanism

The mechanism of the Triton induced hypercholesterolemia in phase I was thought to be due to increased hepatic synthesis of cholesterol through the ability of Triton to interfere with the uptake of plasma lipids by the tissues. Drugs interfering with cholesterol biosynthesis were shown to be active in phase I, while drugs interfering with cholesterol excretion and metabolism were active in phase II[63].

Experimental design

The animals were divided into three groups with six animals in each group.

Group I → Normal control administered with 2% CMC

Group II → Dyslipidemic control received 10% Triton WR – 1339

Group III → Standard administered with Lovastatin (10 mg/kg b.wt) and triton

Group IV → Test drug *Elathy Urundai* - 100mg/kg b.wt.

Group V → Test drug *Elathy Urundai* - 200mg/kg b.wt.

All the animals after 72 hours of triton injection (i.e., after inducing dyslipidemia) the respective treatment was continued for 7 days.

Collection of blood

On the 8th day the blood was collected by retro orbital sinus puncture, under mild ether anesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and it is used for various biochemical experiments. Then animals were sacrificed and collected the liver.

Biochemical analysis

- Total cholesterol
- High-density lipoprotein
- Low-density lipoprotein
- Very low density lipoprotein
- Triglycerides

Anti-Oxidant activity of *Elathy Urundai* (In-Vitro study)

Free radical scavenging activity

DPPH Assay (2, 2-diphenyl -1-picrylhydrazyl)

The radical scavenging activity of extracts was determined by using DPPH assay according to Chang et al [2001].The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10mg/ml DMSO) was used.

Principle

1, 1-diphenyl-2-picryl hydrazyl was a stable free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

$$DPPH + [H\text{-}A] \rightarrow DPPH\text{-}H + (A)$$

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability [64].

Reagent preparation

0.1mm DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

Procedure

Different volumes (2.5µl - 40µl) of plant extracts were made up to a final volume of 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm. 3ml of DPPH was taken as control.

Calculation

$$\% \text{ inhibition} = \frac{\text{Control} - \text{test}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

5. RESULTS AND DISCUSSION

Many studies have been carried out to bring the efficacy and potency of the drug ***Elathy Urundai***. The study includes literary collections, organoleptic character, physicochemical, instrumental analysis, toxicological study and pharmacological study. The drug ***Elathy Urundai*** has been selected for Anti-Diabetic activity in reference with the text ***"Sarabenthirar vaithiya muraigal".***

- Literary collections about the drug from various text books were done. Siddha literatures related to the drug bring the evidence and importance of its utility in treating the diabetes.
- Gunapadam review brings the effectiveness of the ingredients present in the drug for Anti-diabetic, Anti-dyslipidemic and Anti-oxidant properties.
- Botanical aspect explains the identification, morphological description, microscopic character, macroscopic character, active principle and medicinal uses of the plants.
- Siddha and modern aspect of disease explains about clinical features and complication of Diabetes mellitus and also explains adverse effect of drugs used in modern aspect.
- Pharmaceutical review describes about the purification, preparation, administration of *Mathirai* and explains about its self-life properties.
- The pharmacological review explains about the methodology of Anti-Diabetic Activity and the drugs used.

Standardization of the test drug

Standardization of the drug is more essential to derive the efficacy, potency of the drug by analyzing it by various studies. Following are the results of physicochemical. Physical characterization and estimation of basic and acidic radicals have been done and tabulated.

Toxicological results of the drug and pharmacological activity of the drug were derived. Its result has been tabulated and interpretation was made below. Thus it is to give a complete justification to bring the effectiveness of the trial drug *Elathy Urundai.*

Organoleptic character

Table No.6. Physical characterization of *Elathy Urundai*

S.no	Parameter		Results
1	Colour		Brown
2	Odour		Pleasant
3	Taste		Umami
4	Consistency		Hard
5	Shape		Spherical
6	State of matter		Solid
7	pH		4.41
8	Total ash		5.68%
9	Water soluble Ash		2.47%
10	Acid insoluble Ash		1.10%
11	Solubility		
	I	Distilled water	Soluble
	II	Benzene	Soluble
	III	Chloroform	Soluble
	IV	Carbon tetra chloride	Soluble
	V	Xylene	Soluble
	VI	Petroleum ether	Soluble
12	Loss on Drying at 105^0C		4.50%
13	Disintegration time		16 min

Discussion

- pH of *Elathy Urundai* is 4.41 It is acidic in nature[65]. The acidic medium is higher oral bioavailability and was likely to be the result of better solubility and lower clearance. So, the result concludes that the oral bioavailability of the drug is very high [66].
- The amount of minerals and earthy materials present in the drug are represented by Total ash value. The value of *Elathy Urundai*. is 5.68%, it determines the purity of the drug.

- Water soluble ash represents easy facilitation of diffusion and osmosis mechanism. Here the value of Elathy *Urundai* is 2.47% will denote its diffusion capacity.
- The amounts of siliceous matters in the drug are represented by acid insoluble ash value. The acid insoluble ash value of *Elathy Urundai* is 1.10%, which determines the superior quality of the *Elathy Urundai*.
- The moisture content of the drug is determined by loss on drying. These will also indicate stability and shelf life of the drug. Here the percentage denotes the higher stability of the *Elathy Urundai*.
- *Elathy Urundai* is formulated according to classical Siddha text, disintegration indicates the better solubility and absorbability of drug.

Weight variation test

Table No.7. Uniformity weight variation test result of *Elathy Urundai*

S.No.	Weight of each Mathirai (mg)	% of weight variation	Maximum weight variation with in ±7.5%	Maximum weight variation with in ±15.0%
1	138	5.423%	Yes	Yes
2	137	4.660%	Yes	Yes
3	132	0.840%	Yes	Yes
4	130	-0.687%	Yes	Yes
5	139	6.187%	Yes	Yes
6	135	3.132%	Yes	Yes
7	126	-3.743%	Yes	Yes
8	129	-1.451%	Yes	Yes
9	125	-4.507%	Yes	Yes
10	133	1.604%	Yes	Yes
11	127	-2.979%	Yes	Yes
12	128	-2.215%	Yes	Yes
13	136	3.896%	Yes	Yes
14	124	-5.271%	Yes	Yes
15	129	-1.451%	Yes	Yes

S.No.	Weight of each Mathirai (mg)	% of weight variation	Maximum weight variation with in ±7.5%	Maximum weight variation with in ±15.0%
16	131	0.076%	Yes	Yes
17	135	3.132%	Yes	Yes
18	120	-8.326%	No	Yes
19	122	-6.799%	Yes	Yes
20	126	-3.743%	Yes	Yes
	Average : 130.95 mg			

Discussion

- Average weight of the *Mathirai* was noted as 129.855g. Out of 20 tablets tested, 19 tab of them lies within ±7.5% weight variation (1 tab above the limit) and all 20 tab lies within ±15% weight variation.
- According to the limits of weight test cited in the Indian pharmacopoeia, ***Elathy Urundai*** passed the Uniformity weight test.
- The uniformity test resembles uniformal distribution of this tablet helps good absorption and distribution.

Traditional test for pill

Character	Inference
Non sticky on rolling	+
No cracks over the surface after drying	+
Shall be rolled uniformly over the plane surface	+

Phyto chemical analysis

Table No.8. Results of phyto chemical findings of *Elathy Urundai*

Phytochemicals	Test used	Result
Flavonoids	Shinoda test	Positive
Tannins	Gelatin test	Positive
Phenols	Ferric chloride acetic acid test	Positive
Alkaloids	Mayer's test	Positive

Discussion

Phytochemicals are natural bioactive compound found in plants and fibers which act as a defense system against diseases and more accurately, to protect against diseases**.** The phytochemical analysis reveals the presence of alkaloids, tannins, flavonoids, anthral glycosides, cardiac glycosides, saponins, phenols, proteins and carbohydrates.

Flavonoids

- It is the most important group of polyphenolic compounds in plants.
- Flavonoids have potent Anti-Oxidant activity and it is its important function.
- Oxidative stress plays a vital role in the pathogenesis of diabetes mellitus.
- Flavonoids are potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity [67]. Flavonoids can exert their Anti-Oxidant activity by scavenging the free radicals, by chelating metal ions or by inhibiting enzymatic systems responsible for free radical generation.
- It also possesses anti-microbial activity which is confirmed by the various anti-microbial assays.

Tannins

- Reported to have anti-diabetic activity.
- They restore the Anti-Oxidant status of the organs to almost normal levels.
- Increases the cellular Anti-Oxidant enzymes.
- Helps in healing of wounds and inflammation of mucous membrane.

Phenols

- Effective anti-hyperglycaemic agent.
- They possess rich Anti-Oxidant property and protect body from oxidative stress.
- Phenol groups are the essential part of many anti-oxidant compounds.

Alkaloids

- Alkaloids possess antispasmodic, analgesic, bactericidal effects.
- Alkaloids are the active principles producing many essential effects in protecting the body.
- A synergistic effect of all these flavonoids, alkaloids, glycosides, tannins, phenols, increases the potency of the drug against Diabetes mellitus.

TLC Photo documentation : *Elathy Urundai*

Stationary Phase - Silica Gel 60 F_{254}

Mobile Phase – Toluene: Ethyl acetate: Formic Acid (5:1.5:0.15 v/v/v)

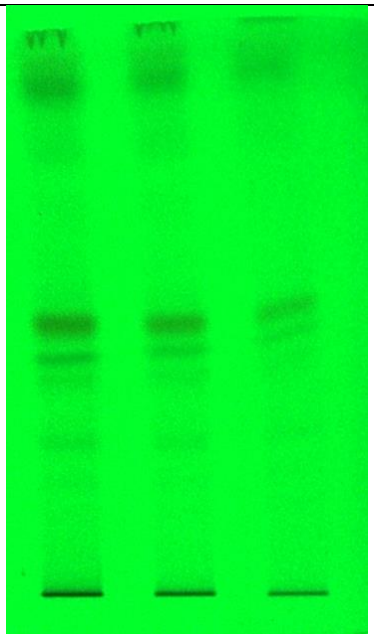
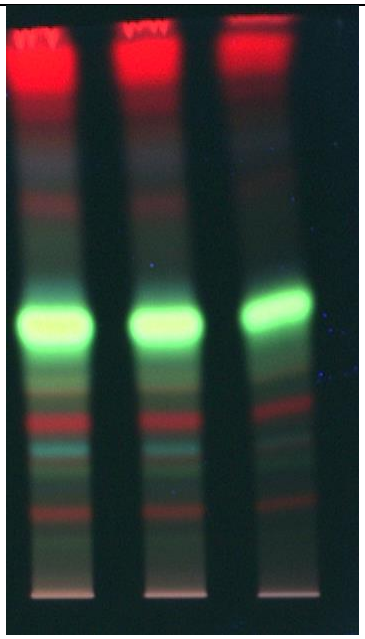
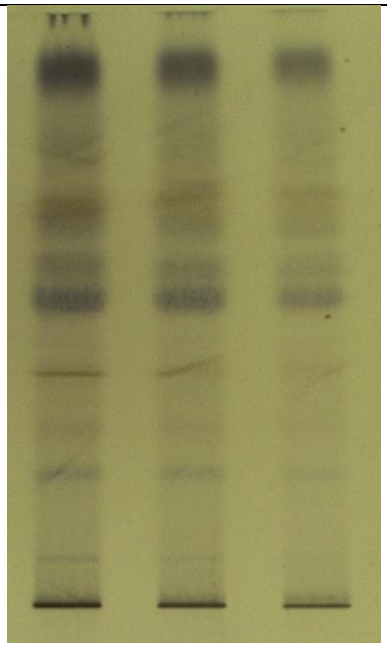
		
λ = 254 nm	**λ = 366 nm**	**λ = 575 nm (Derivatized)**

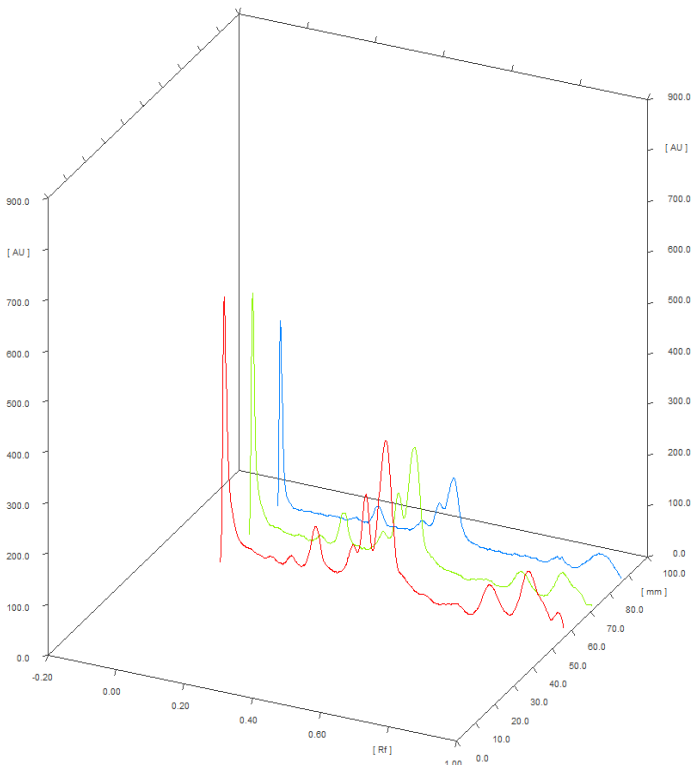
Table No.9. Rf values

Color	R_f value(s)	Color	R_f value(s)	Color	R_f value(s)
Grey	0.21	Green	0.10	Grey	0.08
Grey	0.28	Red	0.15	Blue	0.22
Grey	0.38	Green	0.22	Brown	0.30
Grey	0.42	Red	0.24	Yellow	0.39
Bright blue	0.48	Blue	0.26	Brown	0.51
Grey	0.88	Red	0.30	Black	0.58

Color	R_f value(s)	Color	R_f value(s)	Color	R_f value(s)
		Red	0.35	Violet	0.69
		Green	0.36	Black	0.90
		Bright Green	0.47		
		Red	0.68		
		Red	0.79		
		Red	0.96		

- Under UV 254nm, it shows 6 major spots at Rf 0.21, 0.28, 0.38, 0.42, 0.48, 0.52, 0.88 major compounds are found.
- Under UV 366nm, it shows 12 major spots at Rf 0.10, 0.15, 0.22, 0.24, 0.26, 0.30, 0.35, 0.36, 0.47, 0.68, 0.79, 0.96 major compounds are found.
- Under UV 575nm, it shows 8 major spots at Rf 0.08, 0.22, 0.30, 0.39, 0.51, 0.58, 0.69, 0.90 major compounds are found.

3D Chromatogram of 254 nm:



HPTLC Cromatogram @ 254 nm:

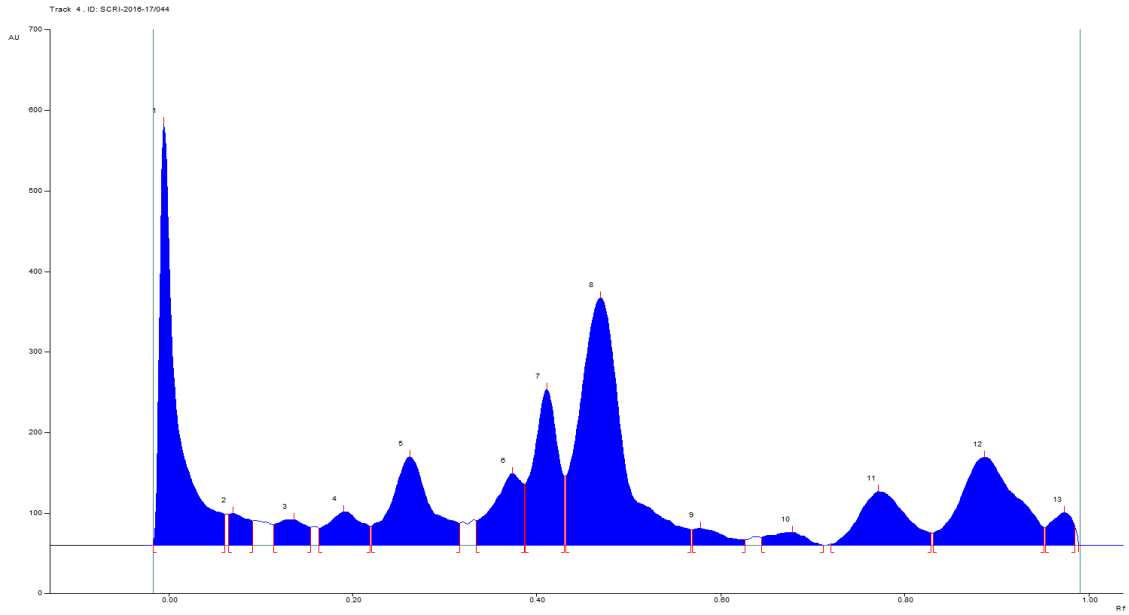


Table No.10. Peak Table @ 254 nm:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	3.5 AU	-0.01 Rf	523.6 AU	32.94 %	0.06 Rf	38.4 AU	8437.7 AU	18.24 %
2	0.06 Rf	39.1 AU	0.07 Rf	39.4 AU	2.48 %	0.09 Rf	30.2 AU	746.2 AU	1.61 %
3	0.11 Rf	25.8 AU	0.14 Rf	31.9 AU	2.01 %	0.15 Rf	22.3 AU	907.6 AU	1.96 %
4	0.16 Rf	21.1 AU	0.19 Rf	41.7 AU	2.62 %	0.22 Rf	23.5 AU	1383.5 AU	2.99 %
5	0.22 Rf	23.8 AU	0.26 Rf	109.6 AU	6.90 %	0.32 Rf	27.5 AU	4181.2 AU	9.04 %
6	0.34 Rf	31.0 AU	0.37 Rf	89.1 AU	5.61 %	0.39 Rf	75.7 AU	2541.2 AU	5.49 %
7	0.39 Rf	76.1 AU	0.41 Rf	193.8 AU	12.20 %	0.43 Rf	85.7 AU	4505.3 AU	9.74 %
8	0.43 Rf	85.8 AU	0.47 Rf	307.3 AU	19.34 %	0.57 Rf	19.2 AU	12709.3 AU	27.47 %
9	0.57 Rf	19.3 AU	0.58 Rf	20.9 AU	1.32 %	0.63 Rf	7.2 AU	637.0 AU	1.38 %
10	0.65 Rf	10.0 AU	0.68 Rf	16.1 AU	1.01 %	0.71 Rf	0.1 AU	557.0 AU	1.20 %
11	0.72 Rf	1.5 AU	0.77 Rf	66.5 AU	4.18 %	0.83 Rf	15.6 AU	3005.4 AU	6.50 %
12	0.83 Rf	15.2 AU	0.89 Rf	109.1 AU	6.87 %	0.95 Rf	22.1 AU	5803.3 AU	12.54 %
13	0.95 Rf	22.3 AU	0.98 Rf	40.2 AU	2.53 %	0.99 Rf	23.0 AU	854.7 AU	1.85 %

Discussion

- The quantitative analysis of compounds present in the EU has been performed by HPTLC. The method may be applied to identify the EU from other manufacturing process. It provides the identification of constituents,

determination of impurities and quantitative determination of active substance present in EU[68].

- The Rf value of the EU supports the better standardization of the drug.
- The present study revealed that EU showed best results in Toluene: Ethyl Acetate (5:1.5) Solvent system. After scanning and visualizing the plates in absorbance mode at 254nm, 366nm and 575nm. Best results were shown at visible light range.
- EU plate showed different colour phytoconstituents of chloroform extract of EU. The bands revealed presence of seven violets, four reds, two greens, one blue, one white and one yellow, bands showing the presence of alkaloids, glycosides, phenols, triterpenes, flavonoids and quinines.

The results from HPTLC finger print scanned for extract of EU. There are thirteen polyvalent phytoconstituents and corresponding ascending order of Rf values start from -0.02 to 0.95 in which highest concentration of the phytoconstituents was found to be 32.94% and 19.34% with its corresponding Rf value found to be 0.12 and 0.89 respectively.

Bio chemical analysis

Basic radicals

Table No.11. Results for Basic radicals

S.NO	Parameter	Observation	Result
1	Test for Potassium	Yellow colour precipitate	+ve
2	Test For Magnesium	White colour precipitate	+ve
3	Test For Sodium	Yellow colour	+ve
4	Test For Zinc	Formation of white precipate	+ve

Interpretation

The basic radical test shows the presence of Potassium**,** Magnesium, Sodium, Zinc and absence of heavy metals.

Potassium

The K+ inwardly rectifier channel (KIR) is one of the two sub-units of the pancreatic islet ATP-sensitive potassium channel complex (IKATP). It has a key role in glucose-stimulated insulin secretion and thus is a potential candidate for a genetic defect in Type II (non-insulin-dependent) diabetes mellitus [69].

Magnesium

- Hypomagnesemia is a common feature in patients with type 2 diabetes.
- Magnesium is the necessary cofactor for many enzymes that play a vital role in glucose metabolism.
- Magnesium has a beneficial effect on insulin action and glucose metabolism [70].
- Mg repletion may play a role in delaying the onset of Diabetes and potentially warding of its complications [71].

Sodium

- Recently sodium glucose cotransporter 2 reabsorbs most of the glucose filtered by the kidneys. Thereby, lowering the blood glucose levels and have been approved as new anti hyperglycemic drug [72].
- A synergistic effect of all these Calcium, Iron, Potassium, Sodium, Phosphorus and Sulphur increases the potency of the drug against Diabetes mellitus.

Zinc

- Zinc is concentrated in the islet cells and was related to the synthesis, storage and secretion of insulin.
- It is essential in insulin action and carbohydrate metabolism.

- Zinc is the structural part of the key Anti-Oxidant enzymes such as superoxide dismutase. Therefore deficiency of zinc impairs their synthesis, leading to increased oxidative stress.
- It improves the glycemic control and lipid parameter with probable movement in Anti-Oxidant status.
- It has a beneficial effect on diabetic neuropathy and nephropathy.

Acid radical

Table No.12. Results for Acid radicals

S.NO	Parameter	Observation	Result
1	Test for Chloride	Formation of white precipitate	Positive

Interpretation

The acidic radicals test shows the presence of Chloride.

Chloride

The Chloride ions are responsible for buffering action and thus it maintains the equilibrium of the cell membrane.

Microbial load

Availability of bacterial and fungal load in *Elathy Urundai*

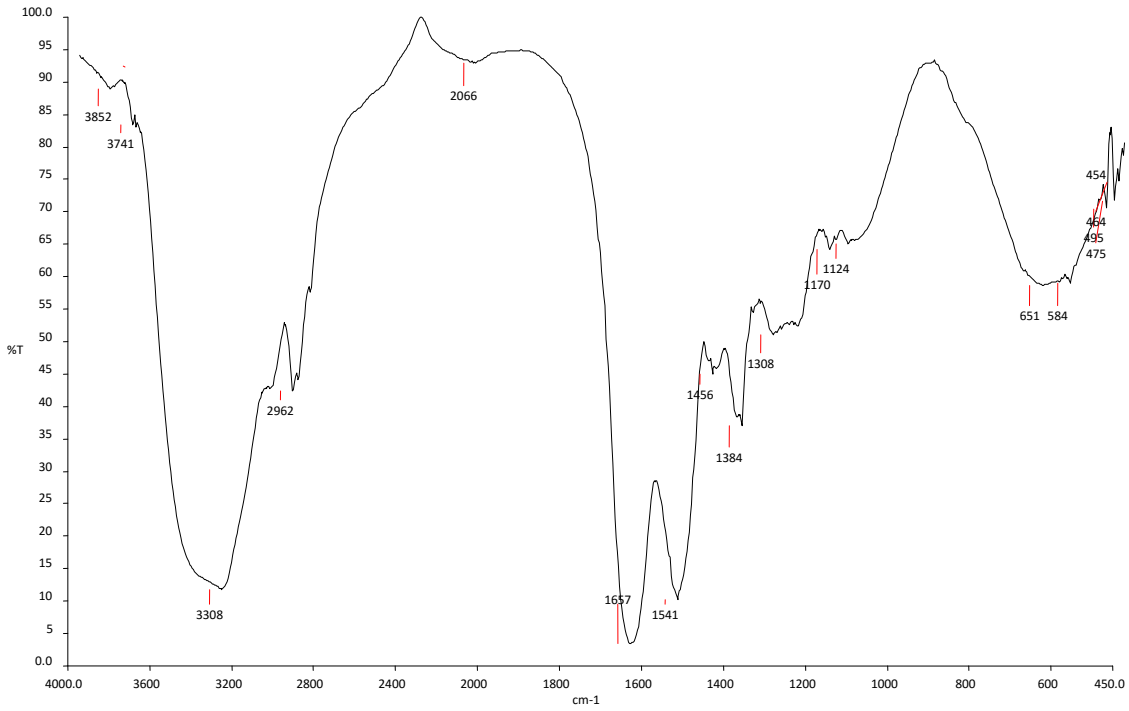
Table No.13. Bacterial and fungal dilutions

MICROBES	DILUTION	RESULT
BACTERIA	**10^{-4}**	**8**
BACTERIA	**10^{-6}**	**6**
FUNGI	**10^{-2}**	**3**
FUNGI	**10^{-3}**	**2**

- The contaminated toxins present in the drug will produce adverse effect, which develops unwanted diseases. They are unfit for humans[73].
- Here, the contamination of *Elathy Urundai* have been examine by bacterial and fungal load.

- Total bacterial load in 10^{-4} dilution is 8 and in 10^{-6} dilution is 6.
- Total fungal load in 10^{-2} dilution is 3 and in 10^{-3} dilution is 2.
- The load of bacterial and fungal are within the limits of WHO norms.

Instrumental analysis



FTIR Spectrum analysis

Table No.14. FTIR Result of *Elathy Urundai*

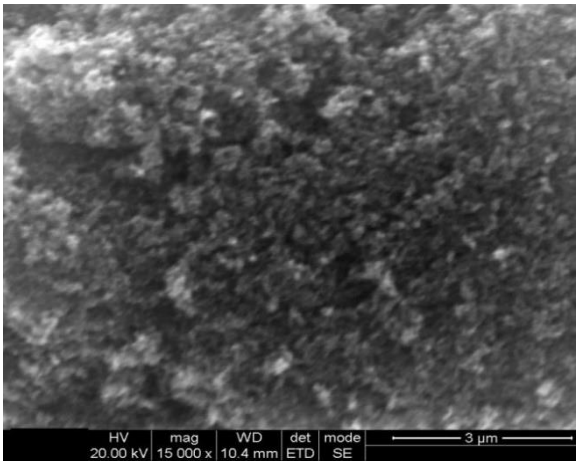
CHARACTERISTIC ABSORPTION(S) (cm^{-1})	**FUNTIONAL GROUPS**
3308	N-H stretch 1º 2º amines ,amides
2962	O-H stretch Carboxylic acids
1657	-C=C-stretch alkenes
1541	N-O a symmetric stretch nitro compounds
1456	C-H bend alkanes
1384	C-H rock alkanes
1308	C-O stretch aromatic amines

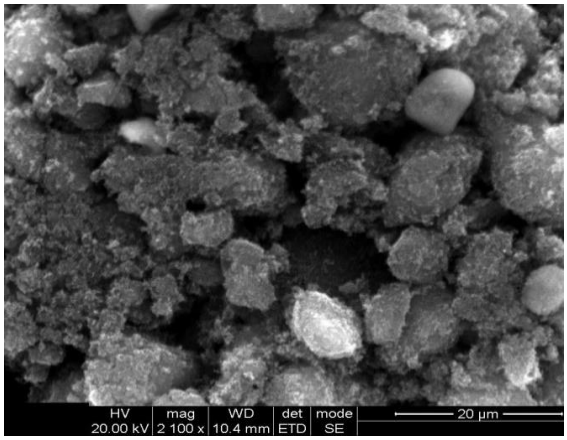
CHARACTERISTIC ABSORPTION(S) (cm^{-1})	**FUNTIONAL GROUPS**
1170	C-H wag(-CH_2X) alkyl halides
1124	C-N stretch aliphatic amines
651	C-Br stretch alkyl halides
584	C-Br stretch alkyl halides

Interpretation

- In FT-IR Spectra analysis, this sample Elathy Urundai exhibits the peak value at 3308, 2962,1657,1541,1456,1384,1308,1170,1124,651,584, having N-Hstretch, O-H stretch,-C=C-stretch, N-O a symmetric stretch, C-H-bend, C-H stretch, C-H wag(-CH2X), C-N stretch,C-Br stretch.
- This indicates the presence of some organic functional groups such as 1, 2 amines, amides, carboxylic acids, alkenes, nitro compound, alkanes, aromatic amines, alkyl halides, aliphatic amines, alkyl halides. The OH group has higher potential towards inhibitory activity against microorganisms.
- Sometimes the presences of Phenols in medicinal plants possess highly Anti-Oxidant property which enhances the drug effect against the disease. For example, the phenolic compound of Hypericum perforatu.

Scanning Electron Microscopy (SEM)



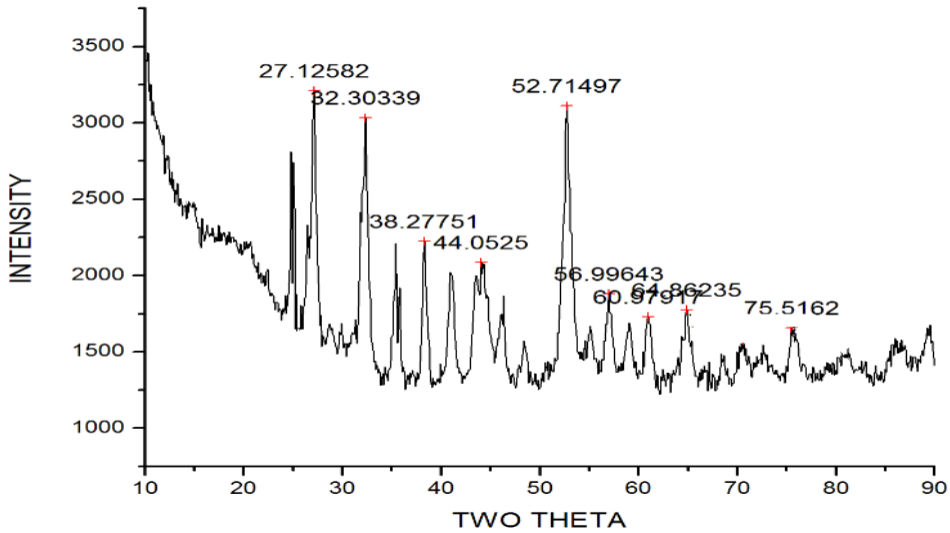


Scanned electronmicroscope image of *Elathy Urundai*

Discussion

- In the above SEM studies of the drug *Elathy Urundai* showed objects of sizes ranging from 3µm to 20µm. The surface of the sample grains is uniformly arranged in agglomerates.
- Size and surface of micro particles can be easily manipulated to achieve both passive and active drug targeting.
- They control the release of drug during the transportation and at the site of localization, alters drug distribution in the body and subsequent clearance of the drug so as to achieve increased drug therapeutic efficacy thereby bio-availability and reduced side effects [74].\

XRD – X ray Diffraction Studies



Interpretation

The structure, the size and shape of the particals are highly dependent on the route of synthesis and high lights the efficacy of the drug. The micro particles may enhance bio absorption of the drug.

The major diffraction peakes are identified after XRD analysis EU concluded that range is 27-52nm associated with organic molecules probably plays an important role in making it biocompatible and nontoxic at therapeutic doses. Other elements present in EU act as additional supplement and possibly helps in increase the efficacy of the formulation.

ICP-OES

Table No.15. ICP-OES Result of *Elathy Urundai*

S. no	Elements	Detected levels
1.	Aluminium	BDL
2.	Arsenic	BDL
3.	Cadmium	BDL
4.	Copper	BDL
5.	Mercury	BDL
6.	Potassium	03.001 mg/L
7.	Sodium	54.300 mg/L
8.	Nickel	BDL
9.	Lead	BDL
10.	Phosphorus	101.311 mg/L

Discussion

- The presence of Potassium (03.001 mg/l), Sodium (54.300mg/l), Phosphorous (101.311mg/l), is physiologically important.
- In *Elathy Urundai*, the heavy metals like Aluminium, Arsenic, Cadmium, Copper, Mercury and Lead were below detectable level. This reveals the safety of the drug.

- From the above results, that the trial drug is safe as it contains heavy metals are observed within the permissible limits.
- Hence the safety of the drug *Elathy Urundai* is ensured.

Potassium

- The K+ inwardly rectifier channel (KIR) is one of the two sub-units of the pancreatic islet ATP-sensitive potassium channel complex (IKATP).
- It has a key role in glucose-stimulated insulin secretion and thus is a potential candidate for a genetic defect in Type II (non-insulin-dependent) diabetes mellitus.

Sodium

- Recently sodium glucose cotransporter 2 reabsorbs most of the glucose filtered by the kidneys. Thereby, lowering the blood glucose levels and have been approved as new anti hyperglycemic drug [58].
- A synergistic effect of all these Calcium, Iron, Potassium, Sodium, Phosphorus and Sulphur increases the potency of the drug against Diabetes mellitus.

Phosphorus

- Phosphorus is needed for the growth, maintenance, and repair of all tissues and cells, and for the production of genetic building blocks, DNA and RNA.
- Phosphorus also need to help balance and use other vitamins and minerals, including vitamin D, iodine, magnesium and zinc.
- Phosphorous was associated with the lower risk of Diabetes mellitus.
- A synergistic effect of all these Potassium, Sodium and Phosphorus increases the potency of the drug against Diabetes mellitus.

TOXICOLOGY STUDIES

Acute oral toxicity in rats– OECD 423

Wistar albino rat was treated with the test drug *Elathy Urundai* of single dose of 2000mg/kg in 2%CMC as suspention. This study was conducted as per the OECD guidelines. The result of acute toxicity of *Elathy Urundai* has been tabulated below.

Table No.16. Observation in acute toxicity study

SL	Group CONTROL	Observation	SL	Group TEST GROUP	Observation
1	Body weight	Normal	1	Body weight	Normally increased
2	Assessments of posture	Normal	2	Assessments of posture	Normal
3	Signs of Convulsion Limb paralysis	Normal	3	Signs of Convulsion Limb paralysis	Absence of sign (-)
4	Body tone	Normal	4	Body tone	Normal
5	Lacrimation	Normal	5	Lacrimation	Absence
6	Salivation	Normal	6	Salivation	Absence
7	Change in skin color	No significant color change	7	Change in skin color	No significant color change
8	Piloerection	Normal	8	Piloerection	Normal
9	Defecation	Normal	9	Defecation	Normal
10	Sensitivity response	Normal	10	Sensitivity response	Normal
11	Locomotion	Normal	11	Locomotion	Normal
12	Muscle gripness	Normal	12	Muscle gripness	Normal
13	Rearing	Mild	13	Rearing	Mild
14	Urination	Normal	14	Urination	Normal

Table No.17. Dose finding experiment and its behavioral signs of toxicity for *Elathy Urundai*

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	**Control**	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	**2000mg**	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1.*Alertness 2.Aggressiveness 3. Pile erection 4. Grooming 5.Gripping 6. Touch Response 7. Decreased Motor Activity 8.Tremors 9 Convulsions 10. Muscle Spasm 11. Catatonia 12.Musclerelaxant 13.Hypnosis 14.Analgesia15.Lacrimation 16. Exophthalmos 17.Diarrhoea 18. Writhing 19 Respiration 20. Mortality*

Body weight

Table No.18. Body weight of wistar albino rats in acute group exposed to *Elathy Urundai*

DOSE	DAYS		
	1	7	14
CONTROL	280.2±42.30	281.4 ± 64.12	282.6 ±26.18
HIGH DOSE	280.4± 21.24	281 ± 3.64	281.4 ± 2

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D(One way ANOVA followed by Dunnett's test)*

Table No.19. Water intake of wistar albino rats in acute group exposed to *Elathy Urundai*

DOSE	DAYS		
	1	6	14
CONTROL	61 ± 1.12	62±2.22	63.9±1.14
HIGH DOSE	62.2±1.1	63±1.14	64.20±24
P value (p)*	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No.20. Food intake (gm/day) of Wistar albino rats in acute group exposed to *Elathy Urundai*

DOSE	DAYS		
	1	7	14
CONTROL	56.24±2.22	56.2±7.42	58.4±3.46
HIGH DOSE	60.6±1.63	60.6±2.62	64.1±5.38

Discussion

In the acute toxicity study, the rats were treated with different concentration of *Elathy Urundai* from the range of 5mg/kg to 2000mg/kg which did not produce signs of toxicity, behavioral changes, and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period.

These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract.

In acute toxicity test the *Elathy Urundai* was found to be non-toxic at the dose level of 2000mg/ kg body weight.

Results of sub-acute oral toxicity 28-days repeated dose study in rats

Wistar albino rat was treated with the test drug *Elathy Urundai* for 28 days repeated dose of 100mg/kg and 200 mg/kg in 2% CMC as suspention. This study was conducted as per the OECD guidelines. The result of sub-acute toxicity of *Elathy Urundai* has been tabulated below.

Body weight

Table No.21. Body weight of wistar albino rats in sub acute group exposed to *Elathy Urundai*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	230.2±15.45	231.5 ± 25.15	231.5 ± 15.50	232.5± 15.16	232.4 ± 15.15
LOW DOSE	230.2 ± 46.22	231.8 ± 32.22	231.6± 66.74	232 ± 62.18	234.8± 54.24
MID DOSE	230.4± 04.24	232.3 ± 46.54	233.2 ± 68.12	234.2 ± 51.26	236.4 ± 54.10
HIGH DOSE	230.6± 64.94	236.6 ± 50.42	249.4 ± 12.24	251 ± 14.38	253 ± 54.61

*NS- Not Significant, **(p > 0.01),*(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Water Intake

Table No.22. Water intake (ml/day) of Wistar albino rats group exposed to *Elathy Urundai*

DOSE	DAYS				
	1	6	14	21	28
CONTROL	50.1 ± 4.32	50±4.12	50.2±1.10	50±1.12	50.4±1.12
LOW DOSE	52.1±1.11	52.8±2.22	52.6±1.42	53.2±2.26	52.4±1.21
MID DOSE	53.1±1.12	53.3±1.11	53.1±2.21	53.4±1.12	53.4±1.42
HIGH DOSE	54.1±1.41	54.2±1.42	54.4±1.44	54.6±1.52	55.8±2.82

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Food Intake

Table No.23. Food intake (gm/day) of Wistar albino rats group exposed to *Elathy Urundai*

DOSE	DAYS				
	2	7	23	22	28
CONTROL	30±5.14	31.2±2.12	31.3±2.18	31.2±1.14	32±2.12
LOW DOSE	30.2±1.14	31.3±1.31	31.1±1.21	31.5±1.32	31.5±1.62
MID DOSE	32.1±2.22	32.2±3.40	32.2±2.24	32.2±2.16	33.2±1.24
HIGH DOSE	33.1±1.12	33.1±1.14	33.6±2.26	34.2±1.10	34.6±3.42

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test.*

Table No.24. Haematological parameters of Wistar albino rats group exposed to *Elathy Urundai*

Category	Control	Low dose	Mid dose	High dose
Haemoglobin(g/dl)	16.7±0.71	16.60±0.24	16.5±0.23	16.82±0.16
Total WBC (×10^3 l)	10.81±0.32	10.64±0.21	10.54±0.42	9.60±1.12
Neutrophils (%)	31.12±0.01	31.02±0.12	32.11±1.22	33.02±6.21
lymphocyte (%)	72.12±1.24	72.12±1.32	73.10±2.34	73.20±2.44
Monocyte (%)	0.9±0.02	0.9±0.01	0.9±0.04	0.9±0.03
Eosinophil (%)	0.5±0.03	0.5±0.04	0.5±0.05	0.5±0.08
Platelets cells10^3/µl	680.17±3.13	682.41±4.12	682.13±2.02	684.10±2.34
Total RBC 10^6/ µl	8.42±0.12	8.46±0.53	8.49±0.44	8.74±0.46
PCV%	42.12±0.2	42.62±1.02	43±1.20	44.40±2.10
MCHC g/Dl	34.5±1.20	34.2±1.10	34.8±1.70	34.33±1.30
MCV fL(µm^3)	58.2±4.02	59.2±1.10	58.9±1.40	58.8±1.20

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No.25. Biochemical Parameters of of Wistar albino rats group exposed to *Elathy Urundai*

BIOCHEMICAL PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
GLUCOSE (R) (mg/dl)	125.11±3.2	125.12±2.10	126.10±13.08	128.12±4.2
T.CHOLESTEROL(mg/dl)	120.16±1.20	120.25±1.30	122.60±1.18	123.24±1.30
TRIGLY(mg/dl)	54.16±1.52	54.12±1.42	56.15±1.23	56.16±1.23
LDL	72.4±2.14	72.12±2.54	73.10±1.32	73.24±10.20
VLDL	11.2±1.30	11.20±2.21	11.22±1.24	11.14±12.14
HDL	27.14±6.12	27.42±2.30	28.16±2.60	28.17±2.14
Ratio 1(T.CHO/HDL)	3.41±1.16	3.42±1.40	3.74±1.04	3.64±2.03
Ratio 2(LDL/HDL)	1.92±1.14	1.91±1.12	1.71±2.20	1.96±08.02
Albumin (g/dL)	5.43±0.16	5.50±0.52	5.04±9.30	5.42±9.48

*NS- Not Significant, **(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No.26. Renal function test of of Wistar albino rats group exposed to *Elathy Urundai*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
UREA (mg/dl)	26.70±0.19	26.50±0.26	27.16±1.28	27.68±1.24
CREATININE(mg/dl)	0.22±0.02	0.21±0.04	0.22±0.05	0.24±0.07
BUN(mg/dL)	17.1±0.01	17.10±0.64	17.6±0.52	17.86±1.02
URIC ACID(mg/dl)	6.04±0.34	6.06±0.51	6.6±0.15	6.42±0.20

*NS- Not Significant, **(p > 0.01), * (p >0.05) , n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No.27. Liver Function Test of of Wistar albino rats group exposed to *Elathy Urundai*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
T BILIRUBIN(mg/dl).	0.07±0.01	0.07±0.02	0.07±0.01	0.07±0.03
SGOT/AST(U/L)	81.14±1.63	81.31±0.02	82.01±1.24	82.64±1.63
SGPT/ALT(U/L)	78.12±1.08	78.21±1.24	78.14±1.26	77.68±0.01
ALP(U/L)	119.21±3.16	119±32.10	119±12.14	120.03±8.32
T.PROTEIN(g/dL)	6.2.10±0.04	6.2±0.11	6.2±0.10	6.4±0.46

*NS- Not Significant, **(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test.*

DISCUSSION

Body weight

The result of the body weight of rats exposed to control and the trial drug of different dose groups exhibited overall mild weight gain throughout the dosing period of 28 days. The quantity of food taken by the animals from different dose groups and the control is comparably normal.

Water intake

There is no significant change in water intake by the animal during the period of study.

Food intake

The weight increase of the animals showed that the intake of food by the animals was good during the period of 28 days study.

Haematological investigation

There was no significant changes were observed in hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV),

Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups. The increase and decrease in the values obtained were all within the normal biological and laboratory limits.

Biochemical investigation

No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits.

Renal function test

The renal function test of the animals shows the normal limits thus the trial drug was safe and not produce any nephro toxicity, thus it suggests that the trial drug was safe for long term administration.

Liver function test

The total bilirubin, bilirubin direct and bilirubin indirect showed that normal range.

Thus the liver function test of Elathy Urundai shows normal in this 28 day repeated oral toxicity.

Discussion

- From histopathological examinations, the slides of animal's organ did not reveal abnormalities.
- From the acute and sub-acute toxicity studies the drug produced some significant changes .But the values were found within normal limits. So the drug *Elathy Urundai* was nontoxic and safe.
- Thus the safety of the drug is revealed so that it can be administered for long time without any side effects.

PHARMACOLOGY STUDIES

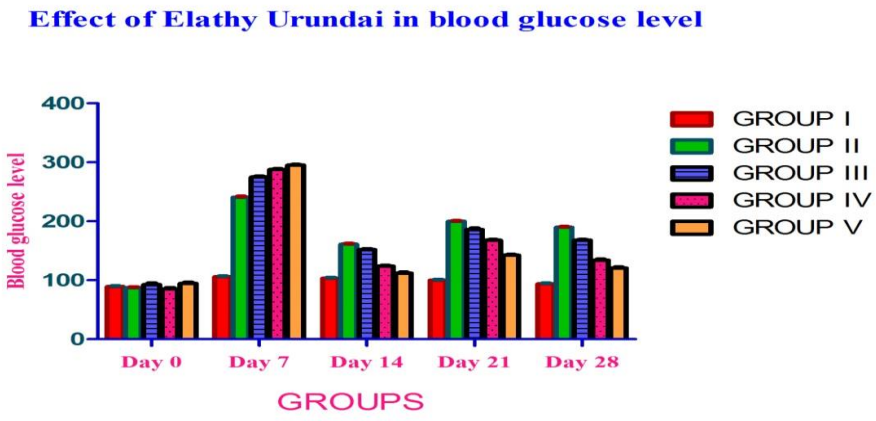
Anti-Diabetic activity

Blood glucose level

Table No.28. Effect of *Elathy Urundai* on Blood glucose level in Streptozotocin induced diabetic rats

Group	Blood glucose (mg/dl)				
	Day-0	Day-7	Day-14	Day-21	Day-28
Normal Control	89.40±1.30	87.53±1.38	92.75±2.86	85.13±2.46	94.70±2.18
Diabetic Control	106.22±1.42	240.9±1.62	275.3±1.46	287.8±1.30	295.4±1.21
Diabetic rats + Glibenclamide	103.5±1.46	161.0±1.34**	152.05±1.3**	124.2±1.43***	112.3±1.46***
Diabetic rats + *EU* 100mg/kg	100.13±1.22	200.12±1.05	186.24±2.17	167.81±1.20**	142.5±1.44**
Diabetic rats + *EU* 200mg/dl	94.23±1.38	189.8±1.34*	168.04±1.28**	134.03±1.70***	120.8±1.83***

*Values are expressed as mean ±SEM (Dunnett's test). *P<0.05 – Significant, **P<0.01 – Highly Significant, ***P<0.001 Extremely Significan*



\ **Chart.1. Anti Diabetic activity of *Elathy Urundai***

Body weight in Streptozotocin induced diabetic rats

Table No.29. Effect of *Elathy Urundai* on body weight in Streptozotocin induced diabetic rats

Group No.	Treatment	Body weight (g) on post induction days			
		Initial	5th day	15th day	20th day
I	Normal control	166.25±2.52	168.89±3.21	169.47±2.58	170.45±2.45
II	Diabetic Control	164.49±2.78	162.80± 3.34	135.80±2.10++	124.30±2.33++
III	Diabetic rats + Glibenclamide	161.40±3.26	164.10 ± 2.77	169.8±3.26	177.30 ± 2.37**
IV	Diabetic rats + *EU* 100mg/kg	166.40±5.78	166.60 ± 3.23	168.80 ± 4.86	172.40± 5.89**
V	Diabetic rats + *EU* 200mg/dl	165.34±6.12	167.42±3.86	168.97±4.99	174.68±6.08**

*Values are expressed as mean ±SEM (Dunnett's test). *P<0.05 – Significant, **P<0.01 – Highly Significant, ***P<0.001 Extremely Significant.*

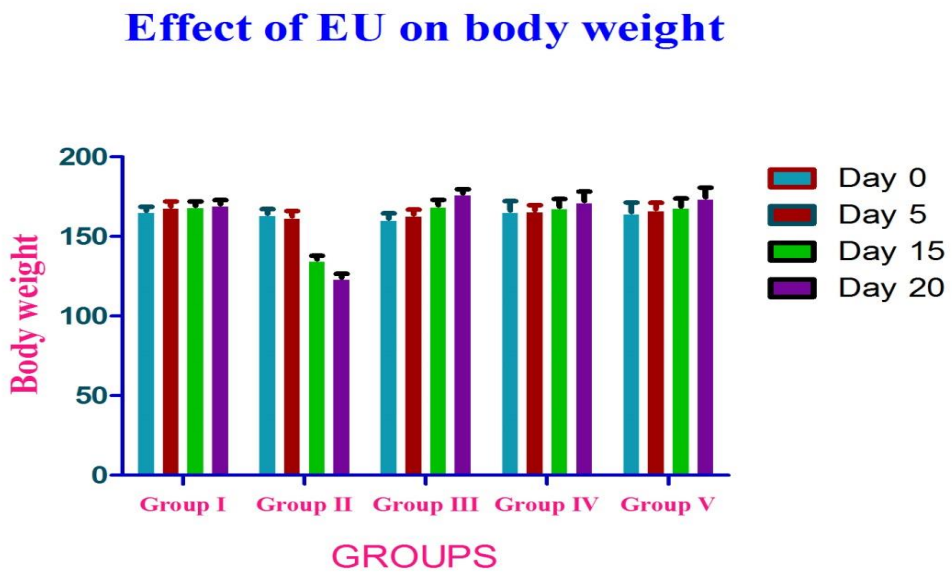


Chart.2.Body weight in Diabetes induced rats

Interpretation

Anti Diabetic activity

The Anti-Diabetic Activity of the test drug Elathy Urundai has been estimated in the streptozotocin induced diabetes in Wistar albino rat.

Administration of the streptozotocin effectively induced diabetes mellitus in the animal model which is known by the increased glucose level.

STZ, slightly cytotoxic agent of pancreatic beta cells, selectively destroys the pancreatic insulin secreting beta cells, thus leaving less active cells and resulting in diabetes mellitus. Thus it is widely used to induce diabetes in animal models. It also interferes with cellular metabolic oxidative mechanisms [75].

Oral administration of the test drug *Elathy Urundai* taken in the dose of 200mg/kg showed significant decrease in the sugar level.

The possible mechanism by which the test drug *Elathy Urundai* brings about a decrease in the blood sugar may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from β-cells of the islets of Langerhans or its release.

Elathy Urundai a Siddha herbal formulation used in the treatment of diabetes shows significant reduction in the sugar level which was studied by inducing diabetes in the animal model Wistar albino rat by streptozotocin.

Body weight changes

Body weight of the animal models in this study was also monitored. There is decrease in the body weight of the animal treated with the control. Whereas the animal treated with *Elathy Urundai* shows a significant improvement in the body weight. Thus the trial drug not only reduced the sugar level but also maintains the body weight in Diabetes Mellitus.

Anti-Dyslipidemic activity

Table No.30. Effect of *Elathy Urundai on* lipid profile of Triton induced Dyslipidemia in rats

Group No.	Lipid Parameters	Total Cholesterol Mg/dl	LDL Mg/dl	VLDL Mg/dl	TGL Mg/dl	HDL Mg/dl
I	Normal control	72.80±1.20	57.43±2.2	19.74±0.60	65.02±1.88	33.23±1.25
II	Triton Control	172.81±7.43	127.80±0.90	81.50±3.55	127.50±1.70	28.30±0.97
III	Dyslipidemic+ Levostatin	63.13±7.3**	50.40± 6.10**	41.50±4.08**	57.20 ± 2.04*	69.90±1.59*
IV	Dyslipidemic + *EU*100mg/kg	72.90±8.4*	52.10±3.20**	51.25±4.86*	56.25±2.40*	73.80±2.49
V	Dyslipidemic + *EU* 200mg/kg	73.50±1.03*	53.28 ± 3.70*	52.67 ± 3.26*	58.74±2.64*	71.21±2.45*

*Values are expressed as mean ±SEM (Dunnett's test). *P<0.05 – Significant, **P<0.01 – Highly Significant, ***P<0.001 Extremely Significant.*

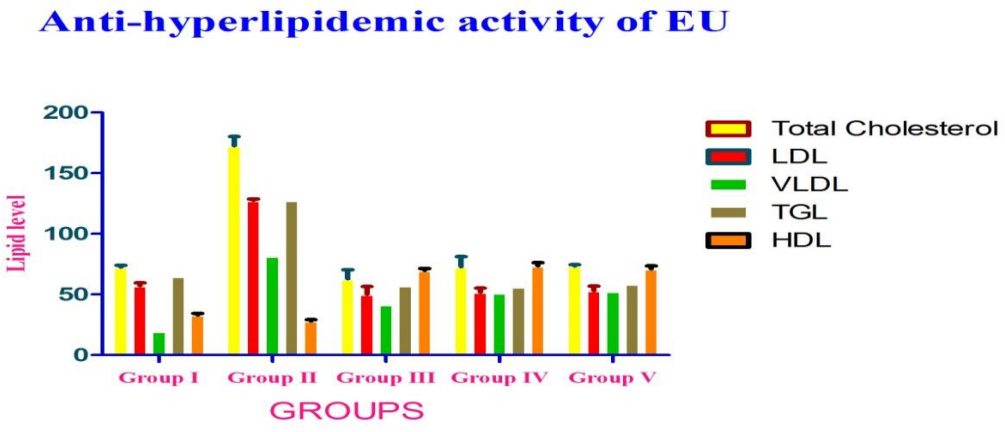


Chart 3:- Anti Dyspipidemic activity

Interpretation

Anti-hyperlipidemic activity of the test drug *Elathy Urundai* was conducted by Triton Wr 1339 induced hyperlipidemia in animal model Wistar albino rats. The results were tabulated above.

The test drug *Elathy Urundai* of 200mg/kg b.wt showed significant changes in the lipid level. There is a significant decrease in the level of LDL, VLDL, TGL. There is a significant increase in the HDL also noted.

The standard drug also showed the significant decrease in the LDL, VLDL, TG. But there is no significant increase in the HDL.

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia, hypertriglyceridaemia and hypercholesterolaemia, resulting from defects in insulin secretion or action or both [76].

In diabetes mellitus along with hyperglycemia there is hyperlipidemia always accompanied representing risk factor for coronary heart disease and other complications.

Deficiency in insulin or insulin resistance may be responsible for hyperlipidemia. Insulin has a inhibiting action on the enzymes involved in biosynthesis of cholesterol.

Therefore deficiency of insulin results in the increase in level of LDL, VLDL, TG, cholesterol and decrease in HDL.

Hyperlipidemia produces further vascular complications and increases the severity of diabetes [77].

The drug showed significant decrease in the LDL which is almost equal to the standard and marked increase in the HDL than the standard drug.

Hence this anti-hyperlipidemic activity supports the anti-diabetic activity of the drug *Elathy Urundai* in giving an effective treatment of diabetes as a whole.

Anti-oxidant activity

Table No.31. Effect on Oxidative Stress by *Elathy Urundai*

Sample concentration (µg/ml)	Absorbance		Percentage of Inhibition	
	Drug	Standard	Drug	Standard
Control	0.5271	0.312	-	-
1.25	0.3873	0.278	26.2103	40.89
2.50	0.3585	0.202	33.2492	51.25
5	0.3365	0.084	60.1038	74.07
10	0.2901	0.052	71.2592	83.33
20	0.2611	0.034	76.5702	89.62

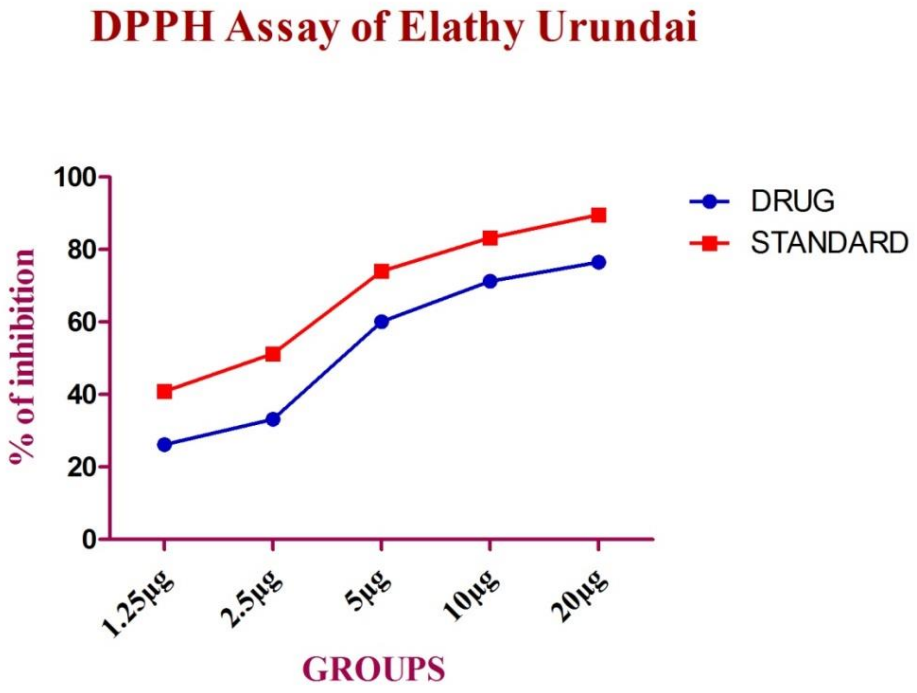


Chart.4. Anti Oxidant activity

Interpretation

Anti-Oxidant study of the trial drug *Elathy Urundai* was conducted and the result has been given in the above table. The trial drug possesses significant Anti-Oxidant property.

Oxidative stress is one of the major pathophysiology of the disease. Oxidative stress is associated with the increased production of reactive oxygen species and impaired antioxidant defense systems, which cause lipid peroxidation, alteration in antioxidant enzymes and impaired glutathione metabolism [78].

In diabetes mellitus tissue damage is brought about mainly by the oxidative stress. There are certain enzymes involved in the Anti-Oxidant mechanism protecting the body from damage. The study shows the amount of the anti-oxidant enzymes increased significantly after the administration of the trial drug thus revealing its Anti-Oxidant property.

Hence the test drug *Elathy Urundai* possesses rich Anti-Oxidant property.

CONCLUSION

6. CONCLUSION

The drug *Elathy Urundai* was selected to validate the safety and its efficacy for Diabetes mellitus in animal model (Wistar albino rats).

The ingredients of the drug was identified and authenticated by Gunapadam experts. The drug was prepared as per classical Siddha literary procedure and subjected to various studies to reveal the potency and efficacy of the drug.

The Organoleptic character and physico chemical studies were made to standardization of the drug *Elathy Urundai.* From the above studies, the *Elathy Urundai* was standardized as per AYUSH guidelines.

The analysis of biochemical, instrumental was made to know the presence of active ingredients in the drug which is responsible for its activity.

Here, the biochemical analysis showed the presence of Potassium,Magnesium, Sodium,Zinc by its synergistic effect, the drug as activity against the disease.

In instrumental analysis, FTIR showed the peak values represents the functional groups responsible for its activity. SEM picture explained the particle size of the drug. In ICP-OES described about the absence of heavy metals and its permissible limits which showed the safety of the drug.

Toxicity studies revealed about the acute and sub-acute toxicity effect of the *Elathy Urundai* in rat models. The drug showed no toxicity and mortality in both acute and sub-acute toxicity. According to OECD guidelines, the haematological, biochemical parameters were investigated. There were no significant changes in the functional behaviour and in the normal values. Thus, it was established the safety of the drug administrated when for long time.

Pharmacological studies were done on the rat model for Anti diabetic, Anti-Dyslipidemic activity and In vitro study for Anti- oxidant activity.

In Diabetes the main pathophysiology is increased oxidative stress which results in the tissue damage and it is the main reason for other complications. The anti-oxidant property of this drug is mainly due to the presence of phytochemicals

(flavonoids, phenols, etc.,) and other active ingredients which involve in scavenging the free radicals and prevents tissue damage and other complications.

In Anti diabetic activity, there was significant decreased blood glucose level and slightly increased body weight in the Streptozotocin induced Wistar albino rats.

In Diabetes, hyperglycemia is always accompanied with the dyslipidemia. Deficiency of insulin results in the increase in level of LDL, VLDL, TGL, cholesterol and decrease in HDL. Dyslipidemia produces further vascular complications and increases the severity of Diabetes. The drug showed significant decrease in the LDL and marked increase in the HDL than the standard drug.

In Anti dyslipidemic activity, showed significant decreased LDL, VLDL, TGL, TC levels and marked increased HDL level in the triton WR-1339 induced dyslipidemic in Wistar albino rat models.

In Anti oxidant activity, there was significant effect of drug when compare to standard in DPPH assay.

Thus by surviving all the above factors, it is concluded that the drug *Elathy Urundai* is safe and potent drug with Anti diabetic and Anti-dyslipidemic activity with rich Anti-oxidant activity. This will support the treatment and management of diabetes and its complications. In treating, the disease with this drug it has a synergistic effect of controls blood sugar level, lipid profile and also reduces the oxidative stress and gives a complete treatment for diabetes and its complication as a whole.

SUMMARY

8. SUMMARY

- From the Siddha literature "***Sarabenthirar Vaithiya Muraigal***" the drug *Elathy Urundai* was selected for its Anti-diabetic, Anti-dyslipidemic and Anti-oxidant activities.
- In this dissertation, Introduction explained about the disease diabetes in Modern aspect, prevalence of Diabetes, mortality and morbidity rate, about the Siddha concept, the disease diabetes in Siddha and role of the drug in treating diabetes.
- The drug was prepared as per classical Siddha literature, the ingredients were identified and authenticated by *Gunapadam* experts.
- In review of literature, many studies were carried out about the botanical aspect and *Gunapadam* aspect of the drugs. Pharmaceutical review enclosed about the preparation of drug and the pharmacological review established the methodologies.
- The drug was subjected to various analysis such as physico chemical, biochemical and also instrumental analysis which provided the active ingredients present in the drug.
- Toxicological study was made according to OECD guidelines showed the study of the drug.
- Pharmacological studies were done to reveal the Anti-diabetic, Anti-dyslipidemic activity in animal models and Anti-oxidant activity of *Elathy Urundai* in DPPH assay.
- Results and discussion gave justifications to prove the potency of the drug.
- Conclusion explained the synergistic effect of all active ingredients and activities that supports the study.
- Thus, the herbal formulation *Elathy Urundai* which is validated for its safety and efficacy for treating Diabetes mellitus, would be a great drug of choice.

FUTURE SCOPE

7. FUTURE SCOPE

The drug *Elathy Urundai* has its own potency in treating Diabetes mellitus in animal model which has been established in this study.

However, the mechanism of action by which *Elathy Urundai* produced its effect on decreasing the blood sugar level in experimental animals need to be evaluated in a scientific manner using specific experimental animal models and also multi-center clinical trials are required to understand the exact molecular mechanisms of action. So it could be used worldwide in treatment of Diabetes mellitus.

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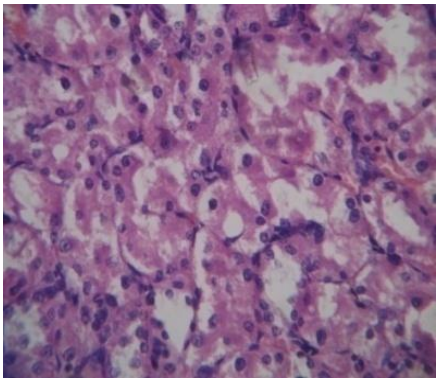
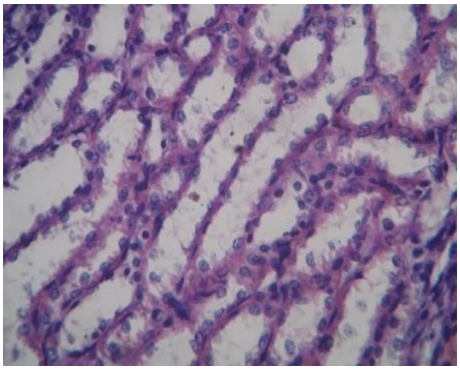
ANNEXURE

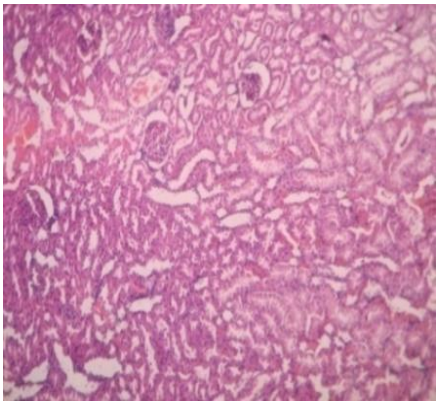
HISTOPATHOLOGY SLIDES OF *EU*

CONTROL GROUP **HIGH DOSE**



Kidney





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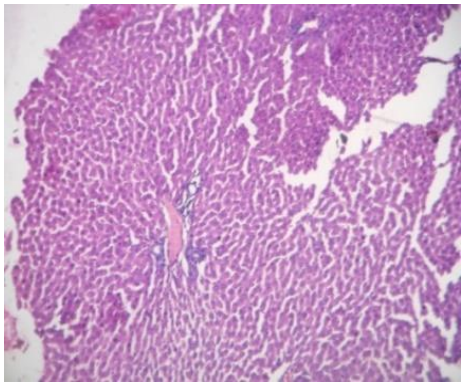
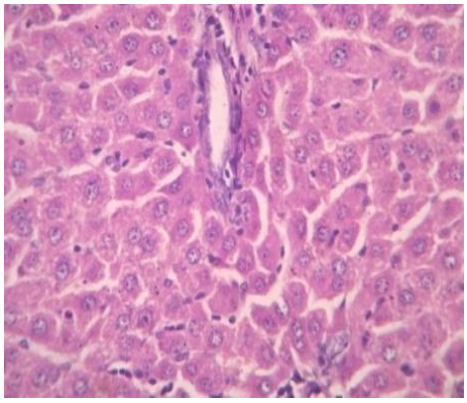
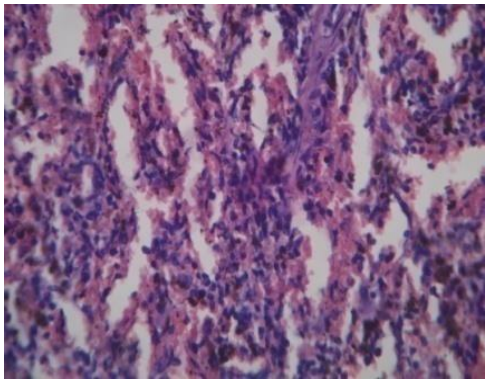


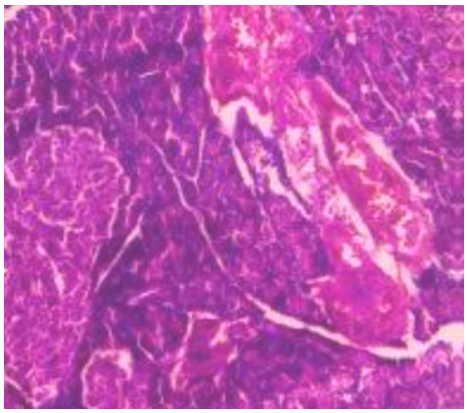
HISTOPATHOLOGY SLIDES OF *EU*

CONTROL GROUP **HIGH DOSE**

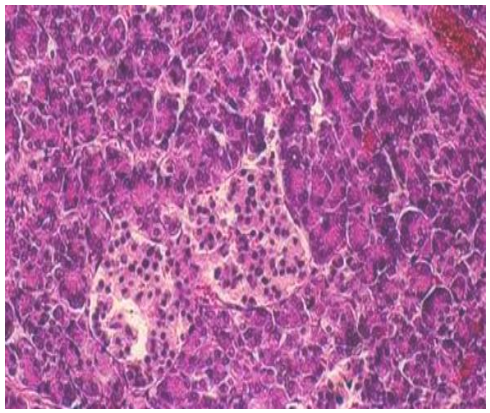


Spleen





Pancreas



PREPARATION OF *ELATHY URUNDAI*

Pound into powder

Grinding with Buttermilk

Rolled pills of *Elathy Urundai*

Cinnamomum tamala
Figure No.3

Cassia auriculata
Figure No.4

Tinospora cardifolia
Figure No.5

Asparagus racemosus
Figure No.6

Nelumbo nucifera
Figure No.7

Butter milk
Figure No.8